

DUODENAL pH:
NEW ASPECTS OF PHYSIOLOGY AND PATHOPHYSIOLOGY

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A thesis submitted for the degree of
Doctor of Medicine
University of Cape Town
November 1987

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To my wife, India,
and my parents

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ACKNOWLEDGEMENTS

The work for this thesis was performed in the Department of Surgery at Ninewells Hospital and Medical School, Dundee, Scotland. It was commenced in October 1985, and completed in November 1987.

I would like to thank, most sincerely, Professor Alfred Cuschieri, Head of the Department of Surgery, University of Dundee, for his extremely valuable advice and support during the undertaking of this work. I am also most grateful to him for teaching me the fundamentals of clinical research, and for allowing me to use the facilities of the his department.

I would like to thank Mr Sami Sadek for the use of his computer software, for writing the necessary modifications in the programmes, and for introducing me to computers.

I received a great deal of help from the following people, and I am grateful for their co-operation:

Mr Robert Wood, Mr Paul Preece and Mr Michael Walker, Senior Lecturers in the Department of Surgery, University of Dundee, and Professor Alfred Cuschieri for allowing me to investigate some of their patients;

Miss Elizabeth Michel, Laboratory Technician, Department of Surgery, for her valuable help in maintaining the microlog receiving units and printing some of the study records, and Mr Angus Reid for preparing the Trasylol test tubes for the plasma samples;

Professor Keith Buchanan, Department of Medicine, Queen's University, Belfast, for arranging the plasma samples to be assayed in his department;

Mr Mark Tulley, Department of Medical Physics, for modifying the receiving units and keeping them fully serviced;

Dr Wynne Carter and Mr David Bryce, Department of Medical computing, for their loaning me the word-processing software;

Dr Peter Baker, Department Of Surgery, for assisting me with the statistical analysis and loaning me the necessary software;

Mrs Catherine Hall, Dietician, for her help in organising the two meals;

The staff of the Medical Illustration Department for the preparation of most of the figures and the illustrations;

my warm thanks to all the volunteers who participated in the study.

In addition, I am most grateful to Professor Lynne Baker, Head of Department of Surgery, University of Natal, Durban, for all he did to make this possible, and for his support.

I would like to extend my appreciation to Smith, Kline and French Laboratories, and the Tayside Health Board for financial support.

This thesis was typed and prepared by the author, on an Amstrad PC1512 word processor, and printed using the laser printer of the Department of Surgery.

Figure 2.1 : After Rune 1981

Figure 3.1 : After product information, Radiometer, Copenhagen.

DECLARATION

I, Craig Alexander Eriksen, hereby declare that the work on which this thesis is based is original (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other University.

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ABSTRACT

The pathogenesis of duodenal ulcer is believed to centre around the presence of gastric acid, yet the exact role that acid plays is poorly understood. Previous investigations of the duodenal pH have been restricted by methodological and technical difficulties, and have, for the most part, only monitored the pH in the short-term.

A new reliable system for long-term (twenty four hour), ambulatory, simultaneous measurement of intra-luminal antral and duodenal bulb pH has been developed. The system comprises two glass pH electrodes, a small portable recording unit and a computer-based system for data storage and analyses. Validation of this pH monitoring system was first performed, and the 24 hour ambulatory profiles of antral and duodenal pH of normal healthy subjects were subsequently recorded. Periods of cephalic stimulation and ingestion of a solid meal were included during the study period. Having established the normal profiles, the investigation was repeated in patients with active duodenal ulcer, off-treatment.

The gastric pH profile was similar of both study groups. There were no significant differences between the fasting duodenal bulb pH and the total 24 hour duodenal acid exposure of the ulcer patients and healthy subjects. Acid peak analysis demonstrated that the duodenal ulcer patients exhibited a defect in the propulsive duodenal bulb motility. Gastric stimulation caused a similar pattern of duodenal acidification in the two groups. These results suggest that gastric acid is not of primary pathophysiological importance in duodenal ulcer disease.

The effects of cephalic stimulation and a meal on plasma gastrin, secretin and somatostatin and duodenal pH were examined in healthy subjects and duodenal ulcer patients. The results showed: vagally-released gastrin is not a significant contributor to stimulation of gastric acid secretion in either health or duodenal ulcer disease; duodenal ulcer patients have excessive basal and post-stimulation plasma gastrin levels but a subset of ulcer patients exists, the "Hypergastrinaemic" patients, who exhibit exaggerated gastrin responses, vagal hyperactivity, a defective somatostatin-induced inhibition of gastrin release and a defect in the "switch-off" mechanism of gastric acid secretion. In addition, the physiological role of secretin in inhibiting gastrin release in Man is questionable. This study reveals new aspects in the physiology and pathophysiology of the duodenal bulb pH.

ABBREVIATIONS

The following abbreviations have been used in this work:

VOL : Healthy subjects, volunteers

DU : Duodenal ulcer

H^RG : Hypergastrinaemic patients

N^OG : Normogastrinaemic patients

MSF : Modified sham feeding, cephalic stimulation

d.o.f. : Degrees of freedom

PRESENTATIONS AND PUBLICATIONS

The following abstracts and papers had arisen from work done for this thesis:

1. Eriksen CA., Sadek SA., Cuschieri A.: Twenty four hour ambulatory dual gastroduodenal pH profiles in health and duodenal ulcer disease. Gut 1986; 27: A1256.

Presented at the Congress of British Society of Gastroenterology, 25 Sept. 1986, Cardiff.

2. Eriksen CA., Buchanan KD., Cuschieri A.: Gastrin responses and duodenal acidity after sham feeding and a meal in duodenal ulcer disease. Presented at the Meeting of Academic Departments of Surgery in Europe, 10 April 1987, Pavia, Italy.

3. Eriksen CA., Sadek SA., Cuschieri A.: The effect of modified sham feeding and a meal on duodenal bulb pH: Evidence of increased vagal tone in duodenal ulcer patients. Europ. Surg. Res. 1987; 19: 56-57.

Presented at the Congress of European Society for Surgical Research, 12 May 1987, Aarhus, Denmark.

4. Eriksen CA., Buchanan KD., Cuschieri A.: Duodenal acidification and gastrin responses after feeding: a new look at the physiology. Gut 1987 (In Press). Poster presentation at the Congress of British Society of Gastroenterology, 18 Sept. 1987, London.

5. Eriksen CA., Sadek SA., Cuschieri A.: 24 Hour ambulatory dual gastro-duodenal pH monitoring: the role of acid in duodenal ulcer disease. Submitted for consideration to Journal of Clinical Investigation.

6. Eriksen CA., Buchanan KD., Cuschieri A.: The effects of modified sham feeding and a solid meal on plasma gastrin and duodenal acidity in normal subjects and patients with duodenal ulceration. Submitted for consideration to Gastroenterology.

CHAPTER 1

DUODENAL pH: STUDY AIMS AND HYPOTHESES

1.1 INTRODUCTION

Although, for the past century, the aetiology of duodenal ulcer disease has eluded all investigators, the presence of gastric acid in the duodenal bulb remains the major determining factor. Amongst the evidence for this, is the very strong association between duodenal ulceration and the gastric hypersecretion seen in patients with the Zollinger-Ellison syndrome (Ellison and Wilson 1964), as well as, apart from rare exceptions (Duberstein and Efrusy 1977), duodenal ulceration does not occur in the absence of gastric acid. This emphasises what Schwarz stated in 1910: "no acid, no ulcer" (Schwarz 1910 cited in Kirk 1981), and the fact that 70-80% of duodenal ulcers heal after two to six weeks of treatment with an acid-inhibiting agent (Lam and Koo 1983, Lam 1984).

It is not understood the precise nature of the role that acid plays in this disease: whether it is the hypersecretion of gastric acid (Rune 1983); whether the exposure to a "normal" amount of acid in the duodenal bulb is prolonged; or whether there is an imbalance of the normal interplay between acid, bicarbonate and other secretions. A further aspect in the aetiology of the disease is the possible presence of a local mucosal defect of the duodenal bulb, or abnormal mucosal cells (Lawson 1987) which makes it susceptible to ulcer formation following the normal acid exposure. The overall picture is, however, somewhat clouded due to the association of duodenal ulcer with many other diseases (Wormsley 1980).

1.2 STUDY AIMS

The aim of this investigation was to develop a reliable computer-based system for continuous long-term simultaneous measurement of antral and duodenal bulb pH. The system was required to be compact, fully portable and comfortable, so that it might be used in as near-physiological conditions as possible.

A computer was employed for data storage and analysis, thus enabling a large volume of raw pH data to be recorded. In this way, many different types of analyses were able to be performed in a manner more accurate and reproducible than by manual techniques. The maximum amount of information was thus extracted from the raw data and a critical detailed assessment of the study area was made.

The equipment used in this investigation included:

1. Two combined micro pH glass/reference electrodes (GK2801C, Radiometer, Copenhagen, Denmark)
2. A portable pH microlog receiving unit (Medical Physics, University of Dundee Medical School, Dundee, Scotland)
3. IBM PC computer with software developed in the Department of Medical Computing, University of Dundee, Dundee, Scotland.

The investigation was set up in four main parts:

PART 1

Development of the system and validation of the methods

The equipment for pH monitoring was selected, and developed for dual monitoring over long periods (twenty four hours). Likewise, a computer-based system with appropriate analysis programmes was

developed to accommodate the dual method of monitoring. The system was assessed in vitro and then in vivo.

STUDY I

Assessment of the normal profile of 24 hour ambulatory dual gastro-duodenal pH

The study sought to avoid the shortcomings of previous investigations, and set out to record, for the first time, a continuous, 24 hour simultaneous profile of the antral and duodenal bulb pH in normal healthy subjects. These profiles would describe the normal fluctuations in daytime and nocturnal gastric acid secretion and the concurrent changes in the exposure of the duodenal bulb to such acid secretion. Included in this study, was the assessment of the effects of the cephalic and gastric phases of gastric acid secretion on the gastro-duodenal pH.

STUDY II

Assessment of the 24 hour ambulatory dual gastro-duodenal pH profile in patients with acute duodenal ulcer

The aim of the study was to record the 24 hour simultaneous profiles of antral and duodenal bulb pH in patients with active duodenal ulcer, off-treatment. The same periods of cephalic stimulation and ingestion of a solid meal were incorporated. The results obtained from the study were compared with those of the healthy subjects described in Study I, and the hypothesis (below) for this study was examined.

Hypothesis I: Abnormal gastric hypersecretion has been documented in duodenal ulcer disease (Baron 1973, Wormsley 1974), and excessive amounts of acid have been noted in the duodenal bulb (Rhodes and Prestwich 1966). More recently, this latter finding has been disputed

(Bendtsen et al 1986). As these studies have only been performed in the short-term, the exposure of the duodenal bulb to acid over 24 hours is thought to be prolonged.

STUDY III

Assessment of the controls of gastric acid secretion in relation to plasma gastrin, secretin, somatostatin and duodenal pH

The hypothesis (below) enabled this study to be set up to assess the effect of the cephalic and gastric phases of gastric acid secretion on the plasma gastrin, secretin and somatostatin, and duodenal bulb pH in healthy subjects and patients with duodenal ulcer. The interrelationships of these three hormones were also examined.

Hypothesis II: Antral pH regulates gastric acid secretion by neural and hormonal mechanisms, and a low pH inhibits further acid secretion and gastrin release (Woodward et al 1954, Befrits, Samuelsson and Johansson 1984). In addition, vagally-released somatostatin inhibits acid secretion and gastrin release (Johnson and Grossman 1971). This latter inhibition is diminished in patients with duodenal ulcer (Harty, Maico and McGuigan 1986). Acidification of the duodenum causes inhibition of gastric acid secretion and gastrin release (Bloom and Ward 1975). It has been postulated that secretin release is impaired in duodenal ulcer disease (Sainz et al 1985).

CHAPTER 2

MEASUREMENT OF DUODENAL pH: A HISTORICAL REVIEW

2.1 INTRODUCTION

The literature is abound with studies of gastric acid secretion in patients with duodenal ulcer, but only more recently has the acidity within the duodenal bulb been studied. The reason for this discrepancy is that the anatomy of the stomach allows for easy acid measurement and the gastric pH is reasonably constant, generally, showing rises only at meal-times. However, the converse applies to the first part of the duodenum.

The ulcer-bearing part of the duodenum is the bulb. It is only a few centimetres long, has a narrow lumen and due to muscular contractions, can be extremely mobile. Within this short length, neutralization of the acid occurs. This process is governed by a number of factors (Figure 2.1): a) the acidity of the gastric contents; b) the rate of gastric emptying; c) the secretion into the duodenal lumen of duodenal bicarbonate, pancreatic bicarbonate and bile; d) the admixture of these secretions with the gastric contents by retropulsion of the former and propulsion of the latter; and e) outward diffusion from the duodenum of hydrogen ions and carbon dioxide (Rune 1981). These processes lead to an extremely steep pH gradient within the duodenal bulb. Rhodes and Prestwich showed there was a difference of almost 3 pH units between the apex of the bulb and the base (Rhodes and Preswich 1966). This has been more recently confirmed by Rune (1981). Due to the intermittent nature of gastric emptying and duodenal secretion, the acidity is not constant and has been shown to fluctuate widely, up to 6 pH units (Rovelstad, Owen and Magath 1952, Aynaciyan and Bingham 1969, Hannibal, Remvig and Rune 1980, McCloy, Vickery and Baron 1980, Hannibal and Rune 1983, Sekiguchi et al 1985). In contrast, the pH within the

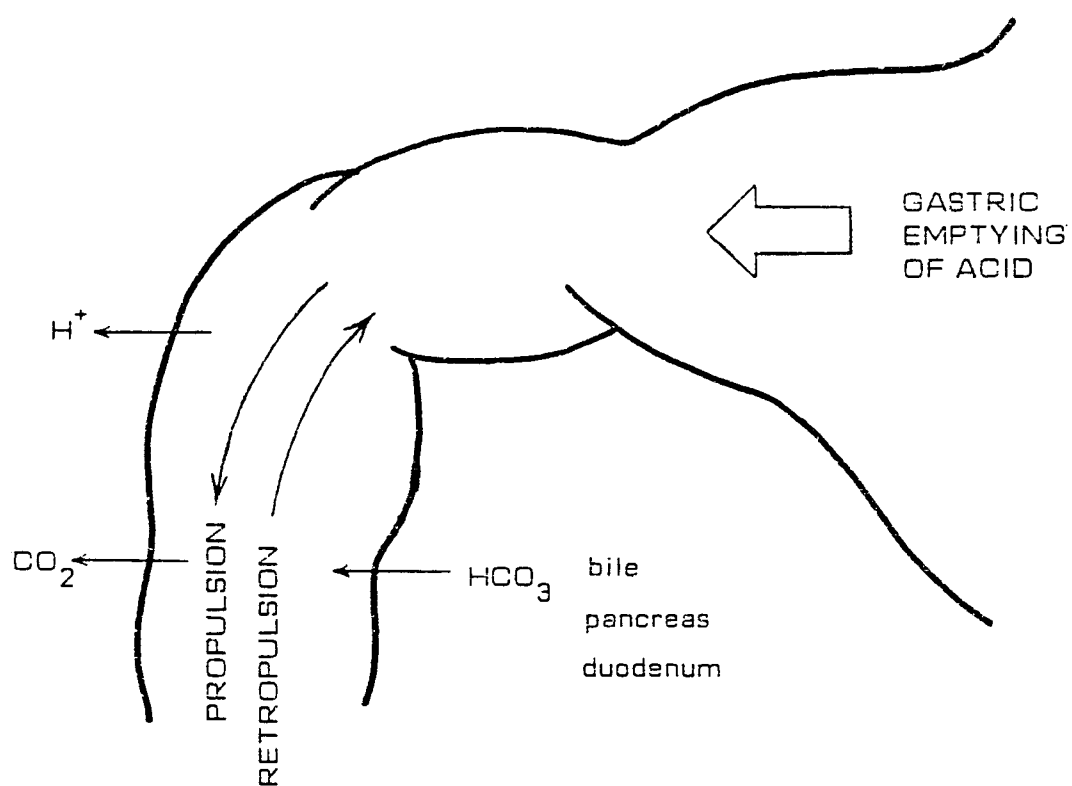


Figure 2.1 : Local factors that govern the neutralization of acid in the duodenal bulb

second part of the duodenum is relatively steady at pH 5.5 - 7.0 (Andersson and Grossman 1965, Archambault, Rovelstad and Carlson 1967, Rune and Viskum 1969).

For these reasons, accurate and reproducible measurements of the duodenal bulb pH have been extremely difficult to obtain. There are three recognized methods of studying the duodenal acidity: namely aspiration techniques with in vitro pH determination; in situ measurement using intraluminal glass pH electrodes; and thirdly in situ measurement with a radiotelemetry capsule ("radio-pill"). Aspiration techniques involve intubating the duodenum with a fine-bore tube. Accurate placement of the tube and especially maintaining its position within the duodenal bulb is very difficult. The negative pressure required to obtain a sample of duodenal content may in fact draw further gastric acid into the duodenum and reflux additional bicarbonate and bile from lower down in the duodenum. This may then provoke further physiological responses and alter the natural situation. A further disadvantage is that this technique is intermittent, as continuous aspiration, for reasons given above, would yield unphysiological results.

In situ measurement using small glass pH electrodes allows far more accurate determination of the acidity. This method was used for the first time in the gastrointestinal tract by McClendon in 1915, when he developed a hydrogen electrode made of platinum with hydrogen bubbling around it (McClendon 1915a, 1915b, 1915c). It was, however, too thick to be placed in the duodenum. Since then, glass electrodes have been designed and perfected. They allow for continuous or very frequent pH measurements in as physiological conditions as possible. For accurate measurements and precise reproducibility, the most

important technical problem, due to the steep pH gradient, is to maintain the electrode at exactly the same position within the duodenal bulb.

The third method of pH measurement is by radiotelemetry as first described in 1957 (Jacobson and Mackay 1957). The pH of the study site is transmitted to a receiving unit. The advantages over the other two methods are improved patient comfort, no possible stimulatory effects as there is no naso- or oro-gastric tube and it may measure acidity at any site of the gastro-intestinal tract. However, the accuracy is in doubt due to technical and developmental problems, and maintaining the position in the duodenal bulb is more difficult than with an in situ electrode.

A detailed review of each of these three methods of duodenal pH measurement is given in the following sections.

2.2 ASPIRATION TECHNIQUES

Examination of the gastric contents was first performed by Kussmaul in the 1870's, and later repeated by such workers as Ewald, Boas and Leube (cited in Einhorn 1910a). As the importance of the role of the duodenum in the digestive process became increasingly evident, attempts were made to study the duodenal contents.

Professor Einhorn, in 1908, constructed a "duodenal bucket" in order to sample the contents of the duodenum, and later used this in the diagnosis of ulcers (Einhorn 1909). However, in the following year, in a paper presented to the Clinical Society of the German Hospital and Dispensary, he alluded to the difficulties encountered whilst measuring

the duodenal aspirate (Einhorn 1910b). His earlier experiences with the duodenal bucket and the aspiration catheter yielded unfavourable results due to both the admixture of the duodenal aspirate with stomach content, and the small volumes that could only be obtained. He constructed an aspiration instrument specific for the task, the "duodenal pump" or "digestive juice aspirator". It consisted of a small perforated metal capsule attached to a thin rubber tube which in turn was connected an aspiration syringe. Confirmation of correct duodenal position was made by the using radiography, aspiration, and the milk test (aspiration performed after three swallows of milk). The resultant aspirate from the duodenum was tested with litmus paper and "usually" found to be faintly alkaline. On two occasions, however, a positive hydrochloric acid (HCl) reaction was obtained in confirmed duodenal contents.

Seven months later, Einhorn reported his results of the duodenal samples from 40 patients with various gastro-intestinal and hepatic disorders (Einhorn 1910a). In these patients, he siphoned the duodenal contents after an initial aspiration rather than using his former technique of continuous aspiration. Five patients were found to have acidic contents; three of these also had free HCl in the duodenum. The samples from the remaining patients showed alkaline reactions on litmus paper, the degree of which varied.

Continuing his investigations with the duodenal pump, Einhorn sought to record the influence of various foods and drugs on the duodenal contents (Einhorn and Rosenbloom 1910). In the fasting state, all 4 patients showed a neutral pH. The ingestion of meat extract or tea produced acidic duodenal fluid in 5 of the 22 tests performed. He later investigated changes in the characteristics of the duodenal

contents that accompanied gall bladder disease (Einhorn 1918) and noted the colour, bile and pancreatic enzymatic content. During this period, McClendon had developed his hydrogen electrode for intraluminal pH monitoring (McClendon 1915a), and he plotted the curves of pH levels in the stomachs and duodena of adults and infants (McClendon 1915c).

The influence of pyloric motility on the duodenal pH was proposed by Myers and McClendon when the latter author swallowed the Einhorn duodenal tube (Myers and McClendon 1920). The first part of the duodenum was studied for three to four hours after a meal. The mean duodenal pH was approximately 7.0, but ranged between 3.20 and 7.82, and sharp fluctuations in the pH from strongly acidic to strongly alkaline were seen. The preponderance of acid peaks were thought to be due to the intermittent spurting delivery of gastric contents into the duodenum. This was confirmed in later years by Bircher et al (Bircher et al 1965) using simultaneous cinefluorography and pH monitoring with an in situ glass electrode. The same authors (McClendon et al 1920) went on to investigate the rest of the small bowel to dispell the "prevalent erroneous impression that the intestinal content is alkaline". With the duodenal tube in situ for 4 days, they reported the pH to range from 4.1 to 6.5. Fifteen years later, a paper from Philadelphia reported a more extensive investigation into the analysis of small bowel contents (Karr, Abbott, and Sample 1935) and showed similar results. Using a double-lumen tube, they studied the whole of the small bowel. The duodenal pH varied between 4.7 and 6.5, and specimens of pH 4.8 were obtained on occasions as far distally as the ileum. The exact site of aspiration within the duodenum was not, however, noted.

The technique of fractional examination of the duodenal content was performed by Einhorn in 1921 when he aspirated the duodenal fluid of 40 peptic ulcer patients every 30 minutes for a two hour period following a test meal of meat extract (Einhorn 1921). This method of sampling and analysis was also employed in two other studies of the same time (Friedenwald and Sindler 1921; McClure, Montague and Campbell 1921). Of the 19 patients with duodenal ulcer, 8 (42.1%) were found, intermittently, to have acidic duodenal aspirates. Four patterns of fasting and post-prandial duodenal pH emerged to subdivide the patient population, and ranged from always alkaline to always acid. It was thought that the prevalence of acidity in the duodenum was due to the fact that some peptic ulcer patients suffered from a disorder of acid neutralisation.

Following on from the work of Myers and McClendon, Okada and Arai examined 15 patients (none of whom had duodenal ulcer disease) while fasting and after various meals (Okada and Arai 1922). The post-prandial time interval ranged from half an hour to six hours, and an Einhorn duodenal tube was used for the aspiration. The fasting duodenal pH varied from pH 6.59 to 7.90, and dropped to 4.80 - 5.97 during the first two hours after eating. Thereafter, the pH returned to vary between 6.05 and 7.95. Free hydrochloric acid (pH <4) was not encountered. They concluded that there was no special relationship between the acidity of the gastric and duodenal contents. A further study regarding the effect of a meal on duodenal pH was carried out in Boston (McClure, Montague and Campbell 1924). The responses to protein (edestin), fat (olive oil) and carbohydrate (arrowroot starch) and a mixture of these was determined in four volunteers. The duodenum was siphoned through a duodenal tube, the tip of which was positioned in the proximal end of the second part of the duodenum, and

the pH of the sample was determined externally using a hydrogen electrode. The fasting duodenal pH was found to range between 6.7 and 8.1. The meal produced very little change in the pH which ranged from 6.0 to 7.8, and thirty minutes after the meal, between 6.0 and 6.8. The ingestion of protein produced an acidic duodenal sample, in contrast to the alkaline pH that was associated fat or carbohydrate ingestion.

One disadvantage of the Einhorn duodenal tube, was the long time required to intubate the duodenum, and then to maintain this position. On occasions, it took up to two hours or longer. Morton reported a low rate of successful duodenal intubations using a Rehfuß tube in patients with duodenal ulcer (Morton 1929). He was only able to pass the tube through the pylorus in only eight of his twenty three patients. Because of this, Richards designed a modified tube and described its use with a somewhat altered intubation technique (Richards 1929). He claimed that adequate sampling of the gastric, duodenal and biliary secretions was possible in half an hour, and fluoroscopic localization was not necessary. However, there are no readily available subsequent reports in the literature describing the use of this modified duodenal tube.

The pathogenesis of peptic ulcer disease was investigated experimentally in normal dogs by Morton, in 1927. He demonstrated the formation of peptic ulcers at sites in contact with gastric acid following the withdrawal of alkaline secretions. He concluded that there was some relative balance at the pylorus between the acidity of the stomach and the alkalinity of the duodenum. With these results, he went on to study patients with and without peptic ulcer (Morton 1929). Two Rehfuß aspiration tubes were used; the first in the duodenum,

the second in the stomach. The positions were checked by fluoroscopy and maintained by keeping the patient lying on his right side. A Ewald test meal of two slices of bread and a glass of water was served and aspirations made for approximately one hour afterwards. The results were reported not as pH but rather as "acidity per cent". The duodenum of the control group was found to be of low and relatively constant acidity, whereas wide variations in the amounts of free hydrochloric acid were encountered in the stomach. In contrast, patients with peptic ulcer showed higher and wider variations of duodenal acidity, as well as the presence of free hydrochloric acid in the duodenum. The gastric acidity of these patients was similar to the control group. The absence of free hydrochloric acid in the duodenum of the normals confirmed the hypothesis that complete neutralisation of gastric acid normally occurs in the duodenum, and there appeared to be a relative acid-alkali imbalance in patients with peptic ulcer, seemingly due to an abnormality of the pylorus. Five years later, Morton confirmed the relationship between pyloric dysfunction and duodenal ulcer disease (Morton 1934). In a study with dogs, he showed that experimentally produced pyloric dysfunction was associated with the subsequent development of duodenitis.

The importance of acid in the pathogenesis of peptic ulcer disease prompted Winkelstein to propose that gastric acid was most harmful when undiluted by other secretions and foodstuffs (Winkelstein 1935). He studied nocturnal interdigestive gastric secretion of 169 subjects (of these, 20 were controls and 62 were duodenal ulcer patients). The group of normals exhibited small secretion rates, and 45% (9/20) had no detectable free hydrochloric acid. In sharp contrast, 92% of the duodenal ulcer patients showed abnormally high gastric acid secretion. The two peaks of acidity amongst these patients occurred at 9 pm and

1 am. and in six out of eight duodenal ulcer patients this nocturnal acidity was not reduced by various pharmacological agents.

Kearney and colleagues noted the infrequent sampling rates of duodenal aspiration recorded in previous studies, and the lack of correlation to gastric acidity (Kearney, Comfort and Osterberg 1941). In their study of seven normals and thirteen duodenal ulcer patients, they used a technique of continuous aspiration of the second part of the duodenum with pH determinations made externally by a glass electrode at two minute intervals. They were thus able, for the first time, to plot the pH profile within the duodenum. The control group experienced 18.2% of the total duration of recordings at pH 4 or below, whereas this figure was 70.2% in the patient group. The authors were able to show that, in normal healthy volunteers, the neutralisation of acid within the duodenum was both prompt and efficient, and the amount of gastric acid entering the duodenum did indeed influence the pH of the latter. However, their patient group showed a far more acidic duodenum, and the longer duration of acid peaks was evidence of a lack of the same duodenal secretory response as seen in the normals. They proposed that the more acidic duodenum found in ulcer patients was due to a) a larger acid load from the stomach, b) a deficiency of "neutralising fluid", and c) a defective duodenal secretory response.

In 1942, Berk and co-workers (Berk, Rehfuess and Thomas 1942) re-emphasised the importance of acid in the aetiology of peptic ulcer disease, and stressed the need to investigate the simultaneous acidity of the "ulcer-bearing area" of the duodenum and that of the stomach. In a group of 21 normal subjects, they used a specifically designed double lumen tube to simultaneously aspirate, at ten minute intervals, contents from the duodenal bulb and pyloric area. The study included

half-an-hour in the fasting state and two hours after an Ewald test meal (toast and water). The mean intragastric pH ranged from pH 2.1 to 3.9 (this low level of acidity was explained on the basis of their inclusion of individuals with very low acid secretory levels). The average fasting duodenal bulb pH was 5.6 (range 1.7-8.3), and dropped to its lowest point of pH 3.8 (range 2.2-6.8) fifty minutes after the meal. However, 31.7% of the recordings were found to be below pH 3.5 (the level the authors adopted for titratable free acid). They concluded that the duodenal bulb is normally an acid area; the neutralising ability in this area is ineffective at some time after a meal; and gastric acidity is not a reliable index of duodenal bulb pH.

Further investigation of dual gastro-duodenal acidity was reported from the same centre (King, Comfort and Osterberg 1944). They used the same double-lumen tube and continuous aspiration technique to study the effects of various drugs on the gastric and duodenal acidity in three normal volunteers. The mean duodenal pH was higher than in their earlier study and varied between 7.53 and 7.70. The intragastric pH was lower and ranged from 1.50 to 1.70. On aspiration of the duodenal content alone, allowing for its admixture with entering gastric juices, the pH dropped to a mean of 5.53. Intravenous secretin produced the greatest increase in the volume of duodenal contents, and raised the pH to 8.45. This latter finding stimulated thought of the possible value of secretin in the treatment of duodenal ulcer, a point taken up 22 years later by Grossman (Grossman 1966).

Long-term, 24 hour, simultaneous aspiration of gastric and duodenal bulb content enabled Atkinson and Henley to demonstrate, for the first time, the temporal relationship of the gastric and duodenal acidities (Atkinson and Henley 1955). Mean intragastric acidity was higher

during the day in patients with duodenal ulcer, compared to normals, but nocturnal levels were slightly lower. Although the acidity of the duodenal bulb was higher than expected, it was not found to decrease at night.

Although, by the mid-1960's, reliable glass electrodes had been designed for in situ pH measurements, aspiration techniques were still frequently used. Rune, from Copenhagen, assessed the value of the post-prandial acid-base status in the duodenum for investigations of the acid neutralisation processes (Rune 1972). Aspirations from the second part of the duodenum showed a significantly lower mean pH in duodenal ulcer patients during the early post-prandial period compared to normals, and the significantly higher P_{CO_2} levels in the ulcer patients showed a positive correlation with both the basal and peak acid outputs. He concluded that the neutralisation capacity of the duodenum in ulcer disease was not defective.

2.3 ELECTRODE TECHNIQUES

At the same time as the aspiration catheters and techniques were being refined, advances were made in designing an electrode to be used for pH measurements of the gastrointestinal tract. The first description of in situ pH measurement of the gastric content was made in 1915 by McClendon (McClendon 1915a). He initially designed a hydrogen electrode for measuring the hydrogen ion concentration of blood and other body fluids. It consisted of a platinum foil and a small U-tube of glass through which hydrogen was bubbled. He modified this electrode so that it could be swallowed and used to determine the pH of either the gastric or duodenal contents. The results, however, were not as accurate as those obtained from larger electrodes, and reported

the pH of gastric juice as being "not far on the acid side of 4". The measurements were recorded as hydrogen ion concentration and then converted into the more convenient pH notation, although McClendon had also designed a potentiometer that was able to read the pH directly (McClendon 1915b). Using this electrode, he was able to draw the acidity curves for the stomach and duodenum in adults and infants (McClendon 1915c). Sampling of the duodenum revealed a pH of approximately 8.

Almost 25 years later, modification of an existing glass electrode was made use of by Eyerly and Breuhaus for investigation into the relationship between gastric acidity and protein digestion (Eyerly and Breuhaus 1939). The electrode was relatively small, 2.75 cm. long and 10 mm. in diameter, and had a liquid junction of saturated potassium chloride solution to the calomel reference electrode. The end of this liquid junction was placed 10 mm. above the glass bulb of the electrode, which was covered by a perforated hard rubber cap. The presence of this shield around the bulb was found to produce a considerable lag in the pH response of the electrode. Later, the necessity of this cap became the subject of some dispute and was finally dropped. Alongside the electrode was placed an aspiration tube, and although no constant difference was encountered between the pH results by aspiration and from the electrode, it was felt that aspiration samples were not sufficiently accurate. In the following year, Eyerly made a further minor alterations to the electrode design (Eyerly 1940). A soft rubber shield replaced the firm one and, 4 cm. distally, a small metal weight was attached to the assembly. The weight facilitated entry into the duodenum and acted like an anchor to maintain the position. This type of design provided the most physiological method of in situ pH measurements and has been adopted,

with minor modifications, for present day studies. Eyerly studied the gastric antrum, duodenal bulb and distal second part of duodenum of two normal volunteers, and the pH was measured every 5 minutes over a two hour period. The fasting pH in the bulb and second part were 1.30, 1.60 and 5.47, 5.96 respectively, and frequent marked swings in pH were noted. These fluctuations were of particular interest and were found to occur both in the fasting state and during antacid therapy. Of the various antacid preparations studied, magnesium trisilicate and aluminum hydroxide yielded the greatest pH rise. Later studies (Rovelstad, Owen, Magath 1952; Rovelstad and Maher 1962) were unable to confirm the magnitude of these pH rises.

The earlier studies utilized the calomel reference cell positioned on the skin of the patient, usually the finger. This, however, produced fluctuations in the measured pH of up to one pH unit due to differences of potentials between skin and the gastro-intestinal tract, and it was open to varying and unmeasured errors (Rovelstad, Owen and Magath 1952; Andersson and Grossman 1965). Using a shielded glass electrode and both cutaneous and enteric reference cells, Rovelstad, Owen and Magath (1952) showed that more accurate results were obtained by placing the reference cell alongside the electrode in the stomach. The glass electrode was preferred as the hydrogen electrode was found to alter the composition of the test solution and the antimony electrode, described earlier (Kreiter and Pantlischko 1949), required elimination of dissolved oxygen and laborious standardization. Rovelstad went on to study the in situ gastro-duodenal pH in 21 patients with and 23 without duodenal ulcers (Rovelstad 1956). He selected the mid-point of the second part of duodenum and found that, although both groups demonstrated similar mean fasting duodenal pH levels, administration of histamine produced a

significantly more acidic duodenal milieu in the ulcer patients than the non-ulcer patients. He concluded that ulcer patients suffered a defective neutralization mechanism.

A further study from the same centre reported on the effect of diet, antacids and anticholinergics on intragastric and intraduodenal pH in patients with duodenal ulcer (Rovelstad and Maher 1962). Two unshielded glass electrodes were used on this occasion. The fasting duodenal pH was approximately 6, with only minor variations, and a meal of bland food produced a significant fall in the pH to below 3.5. This occasionally decreased further to pH 1.5, within 15 to 90 minutes of eating. Wide fluctuations were noted and these were considered to be true pH changes, not artifactual recordings of an unshielded electrode. Milk and antacids elevated the post-cibal pH to above 5, whilst anticholinergics also raised but stabilized the intraduodenal pH. This latter finding suggested the influence of the gastric emptying rate on duodenal pH; the hypothesis being strengthened by the results from the same centre (Bircher et al 1965) shown by simultaneous cinefluorography and pH monitoring.

Being concerned about the accuracy of the pH recordings, Tomenius and Williams modified existing methods and used this to investigate the efficacy of some antacids (Tomenius and Williams 1960). Although the advantages of having the reference electrode in close proximity to the intra-luminal pH electrode had previously been described, the inside of the patient's mouth was selected as the site of the reference cell. A slight change in the patient's position produced a change in the pH reading. As the mucous membrane of the gastro-intestinal tract had been shown to be of approximately pH 7.0 (confirmed again later in 1965 by Bircher et al), the unexpected fluctuations of recorded pH

were thought to be due to the contact of a bare electrode with the luminal surface of the gastro-duodenal region. For this reason, the glass tip of the pH electrode was shielded by a rubber cap. In contrast to earlier studies, their preliminary experiments showed that such a shield did not hinder the recordings of the electrode and did not clog with mucus. This question of whether the electrodes should or should not be shielded was taken up five years later by Rovelstad's group (Bircher et al 1965). In the study of first 4 dogs and then 31 duodenal ulcer patients, the pH of the duodenum was recorded by two pairs of bare and shielded electrodes. The bare electrode showed "inconsistent" and frequent pH fluctuations which seemed to be associated with respiration, heart beat and peristalsis. However, the range of duodenal pH was noted between 1.0 and 7.5. The shielded electrode, on the other hand, produced a narrow pH range which was low and stable, and in agreement with the results of Tomenius and Williams (Tomenius and Williams 1960). This led the authors to believe that more meaningful results were obtained with these electrodes rather than with the bare unshielded electrodes. This, however, appeared in contradiction to their earlier beliefs (Rovelstad and Maher 1962). The vote for shielded electrodes was further endorsed by Andersson and Grossman who also felt that bare unshielded electrodes produced artefactual recordings (Andersson and Grossman 1965).

Previous studies had not been able to identify the position of the pylorus, as no manometrically defined area could be found and the wide fluctuations in measured pH were unsatisfactory. The previously recorded low levels of bulbar pH (Tomenius and Williams 1960, Rovelstad and Maher 1962) were considered doubtful due uncontrolled maintenance of the electrode's exact position within the duodenum.

Andersson and Grossman developed a method of measuring intraluminal electrical potential difference (PD) and identified the location of the gastro-duodenal junction by changes in the transmural PD. A well defined change in the PD to a more negative value was observed as the duodenum was entered (Andersson and Grossman 1965). They also measured the duodenal pH with shielded electrodes and found a relatively steady pH range (6.5 - 7.5) with less than one pH unit of fluctuation. The pH of the duodenal bulb, however, was more closely related to the antral pH except when the latter showed a pH of above 3.5. They also noted that a steep pH gradient existed between the bulb and the rest of the duodenum. This latter finding was elegantly documented a year later by Rhodes and Prestwich (Rhodes and Prestwich 1966).

In a detailed study using the antro-cutaneous and duodeno-cutaneous transmucosal potential differences, Archambault and co-workers were able to accurately locate the pylorus (Archambault, Rovelstad, Carlson 1967). A PD of more than 15 mv indicated the entry into the duodenum.

An alternative method of localizing the exact position of the duodenal bulb and pylorus was reported by Rhodes and co-workers (Rhodes, Apsimon, Lawrie 1966). Using a combination of two glass electrodes, 4 cm. apart, they found that the gastric antrum pH was relatively constant at approximately 2.0, whereas that of the duodenal bulb either fluctuated widely or was nearer neutral. Thus, as the pH tracing changed from being constantly acid, they were able to infer that the distal electrode had entered the duodenum. The positions were checked with fluoroscopy and contrast material outlining the pylorus. Orad traction on the electrode assembly ensured the proximal electrode

remained in the antrum. A small mercury-filled bag was attached to the distal electrode to assist in passage through the pylorus and the effect of peristalsis on this bag maintained a caudal pull on the assembly. In contrast to Tomenius and Williams, they found that the shield around the electrode became blocked with mucus and interfered with the pH measurements. For this reason, they suggested that it not be used. The duodenal bulb pH was recorded over a twelve to eighteen hour period, and included the nocturnal measurements. The post-prandial pH fluctuations, initially small, were increased and irregular at one hour after the meal, and continued for longer in duodenal ulcer patients (up to six hours). With the two electrodes closer together (9.25 cm. apart), Rhodes and Prestwich clearly documented for the first time the steep gradient of pH within the duodenal bulb (Rhodes and Prestwich 1966). The average pH at the base was 3.3 and that of the apex was 5.1. No difference was found between duodenal ulcer patients and controls, although the duration of acid exposure (time $\text{pH} < 2.5$) was longer amongst the patient group. The importance of maintaining the exact position of the electrode within the duodenal bulb became increasingly evident.

Earlier in 1965, Moore and Scarlata eluded to the controversy regarding the lack of a clear relationship between pH and hydrogen ion concentration (free acid) (Moore and Scarlata 1965). They noted that, by definition, the pH related to the negative logarithm of the hydrogen ion concentration, whilst the pH as measured by the glass electrode related to the negative logarithm of the hydrogen ion activity. They introduced a small activity coefficient and were able to demonstrate the accurate conversion of gastric acid pH measurements into true hydrogen ion concentrations. In a subsequent paper, Moore

published an extensive conversion table of pH values into hydrogen ion concentration (Moore 1968).

The fluctuations in duodenal pH were considered to be due to the pattern of gastric emptying. This hypothesis was examined by using air-filled balloons to exert antral pressure (Rhodes, Goodall, Apsimon 1966). It was found that an increase in the antral pressure was usually associated with a drop in the duodenal pH, but for the first $2\frac{1}{2}$ hours after eating, the duodenal pH decreased without any increase in antral pressure. Reflux from the second and third parts of the duodenum was shown to produce an elevation of the bulb pH.

The problems associated with in situ gastro-intestinal pH measurements were becoming increasingly evident. To summarize:

- a) the size of the electrode required to be suitable for in situ use.
- b) the varying membrane potentials across the gastric and duodenal mucosa ranged from -30 to +30 mV and produced errors in the measured pH values.
- c) the pH of the mucosa and that of the intraluminal fluid varied up to 7 pH units.
- d) in view of this latter finding, a shield around the glass tip of the electrode was considered preferential. But the shield was found to clog with mucus and interfered with the pH measurements.
- e) the siting of the reference electrode on the periphery (skin or oral mucous membrane) was liable to produce inaccurate pH readings.
- f) the steep pH gradient within the duodenal bulb necessitated the accurate placement of the electrode and maintenance of this position.

In conjunction with a Danish company and bearing these problems in mind, Rune developed an electrode for in situ measurement of gastro-

intestinal pH (Rune 1968). It was small enough to be passed nasally, and although controversial, had a shield of flexible nylon threads surrounding the glass tip. It also incorporated a potassium chloride salt bridge between the reference electrode and the gastro-intestinal fluid enabling the liquid junction to be close to the glass electrode. This negated the differences in transmucosal potentials. The electrode was able to measure the change in potential between the reference electrode alongside and a second cell placed on the skin. An abrupt 25 - 30 mV change in potential signified the glass electrode's position in the duodenal bulb. Using this electrode, duodenal ulcer patients and controls of similar gastric secretory capacity were studied (Rune and Viskum 1969). It was found that there was no significant difference between the two groups in the fasting or post-prandial duodenal pH, and the frequency of pH fluctuations of the control group was similar to that of the patients. These results appeared to show that duodenal ulcer patients had a sufficient duodenal secretory capacity of bicarbonate, in that the acid neutralization in the duodenum was similar to normals. It is important to note that the second part of the duodenum was studied, and not the ulcer-bearing bulb.

A study from Birmingham (Benn and Cooke 1971) compared the results of shielded and bare electrodes and found no satisfactory agreement between the two sets of readings. On withdrawal of the electrodes, the wire shields were heavily coated with thick mucus and this resulted in a significantly lower pH value when compared to samples tested after aspiration.

A new method of long-term intraluminal pH monitoring was introduced by McCloy, Vickery, Manjil and Baron in 1978 (McCloy et al 1978). They used two glass electrodes in series attached distally to a

weighted bag and employed the principle described by Rhodes and co-workers (Rhodes, Apsimon, Lawrie 1966) to maintain the position within the duodenal bulb. The pH data was logged in digital form, transferred to punched paper tape and analysed on a computerised system. A fuller account of their method was reported two years later (McCloy, Vickery, Baron 1980). The stability of the electrode position was confirmed during a 5 hour test. Unshielded electrodes were used and, although mucus was found to coat the glass tips, the response time was unaltered and changes of 0.04 pH units were encountered after washing. This effectively ended the controversy of the use of a shield around the electrodes. Punch tape was chosen for data storage as, with a sampling rate of every 5 - 10 minutes, it could easily accommodate the numerous recordings. The previously noted peaks of acidity were confirmed and found to last on occasions only a few seconds.

Further use of a computerized system was described in the same year, and the pH sampling rate was increased to be at every half second (Hannibal, Remvig, Rune 1980). This was later elaborated on by Rune (Rune 1982). Further attention was turned to the problem of electrode placement within the duodenal bulb (Hannibal and Rune 1983). They strapped four pH electrodes together at 1.5 cm. intervals and by observing the analogue tracings from these electrodes, they were able to identify the exact positions of each electrode within the "antro-pyloro-duodenal" channel. Fourteen healthy volunteers were studied for 6 to 8 hours to record the pattern of duodenal pH changes. Great individual variation in pH fluctuations were seen, except for during the early post-prandial period. The steep gradient of pH between the proximal and distal bulb was confirmed and became more obvious when the values were converted to mean acidity.

The dual pH electrode system, with a sampling rate of every 10 or 20 seconds, was used in 11 ulcer patients and 8 volunteers in a study investigating duodenal pH changes after a meal, smoking and cimetidine (McCloy, Greenberg, Baron 1984). The mean duodenal pH was similar for both groups, but ulcer patients experienced significantly greater (11.8%) acid exposure at pH <4 than did volunteers (6.2%). Interestingly, the patients showed significantly longer alkalization (63.3%) at pH >6 than volunteers (57.9%). The meal produced the same changes in pH in both groups.

The role of interdigestive gastro-duodenal motility and gastric acid delivery into the duodenum was investigated in an elaborate study from Japan (Sekiguchi et al 1985). They measured simultaneously the antral and duodenal bulb pH and the intraluminal pressure activity at six sites in the stomach, duodenum and jejunum. The motility was shown to be in three phases and the pH fluctuations were related to these phases. Phase I, associated with near neutral pH, was a quiet phase and of shorter duration in duodenal ulcer patients. However, Phase II was considerably longer in these patients and consisted of irregular contractions, and the emptying acid into the duodenum. During this phase, the duodenal pH fluctuated widely. The strong regular antro-duodenal contractions of Phase III were absent in patients with duodenal ulcer.

Two further studies from Rune's unit (Ovesen et al 1986, Bendtsen et al 1986) described the addition of two more electrodes to the four electrode system. Computerized sampling was set at once every second for a digital pH record and simultaneously an analogue reading was obtained. The 3¹/₂ hour study, that included a milk meal, showed no

difference in the intraluminal duodenal bulb acidity and duodenal neutralization between control subjects and duodenal ulcer patients.

The technical and methodological problems associated with duodenal bulb pH measurements were finally being solved. Long-term studies appeared possible and were needed to investigate the normal 24 hour fluctuations in duodenal bulb pH. Armed with such results, the pathophysiological situation in duodenal ulcer disease could be studied.

2.4 pH MONITORING BY RADIOTELEMETRY

The mechanisms within the bladder during micturition stimulated thought of the possibility of a radio transmitter that could be placed within a person to relay the necessary information. Mackay and Jacobson designed such a radio pill, or "endoradiosonde", which being 28 by 9 mm. in size, could easily be swallowed and was found to operate successfully within the gastro-intestinal tract (Mackay and Jacobson 1957). It relayed pressure data by changes in frequency, and temperature data by the rate of transmission of energy pulses. This radio pill was modified with a glass electrode to become the first pH-sensitive telemetry device described (Jacobson and Mackay 1957), and the pH was transmitted as a frequency-modulated signal. The response time was slow and it was of poor accuracy.

Using an antimony electrode as a pH transducer, Noller designed a radio pill which became known as the Heidelberg capsule (Noller 1960). It consisted of a high frequency transmitter and a silver chloride reference electrode. The frequency change was linear over a pH range of pH 2 - 7, and was accurate up to 0.5 pH units. Noller used this capsule as part of the gastric function tests; principally as a

marker of the end point of gastric acid neutralization of intragastrically instilled bicarbonate. Connell and Waters assessed the accuracy and efficiency of the Heidelberg capsule in vitro and evaluated its clinical application (Connell and Waters 1964). They found a good correlation between the values for gastric acid secretion obtained by aspiration 15 - 30 minutes after histamine and those obtained by the pH capsule during the following 15 minutes. It was able to distinguish between high and low rates of gastric secretion and was clearly more comfortable to the patient than a naso-gastric tube. Stack later evaluated the use of the capsule in routine measurement of gastric acid secretion and found that, although it detected extremes of secretion, it was not accurate for minor changes in acid production (Stack 1969).

Watson and Paton, from Glasgow, were the first to describe the use of the Heidelberg capsule for measuring specifically the pH of the small bowel (Watson and Paton 1965). The pH of the proximal jejunum was measured over a four hour period: mean fasting pH was 5.2 (range 3.1 - 6.8); post-prandial pH changes of 0.5 pH units. Maxwell, Ferguson and Watson also reported their experiences in monitoring jejunal pH (Maxwell, Ferguson, Watson 1971). A study from Japan used an untethered radio pill to monitor the pH changes from the stomach aborally through the small intestine (Kitagawa et al 1966).

Three years later, Aynaciyan and Bingham tethered the Heidelberg capsule to a thin tape so as to measure the pH of the proximal duodenum (Aynaciyan and Bingham 1969). They compared the results of duodenal ulcer patients with those of normals and found no

significant differences. The disadvantages of the capsule were the inaccuracy at levels pH <2 and >7, and a longer lag response.

The antimony electrode was found to deteriorate rapidly in the presence of oxidising agents in the body and this made it unsuitable for long-term studies (Colson et al 1981). The glass electrode was more accurate and stable, and had an in vivo operational life of one month. The problem of signal loss was overcome with the use of 2 or 3 orthogonal aerial loops and an aerial switching unit. Watson commented on this latter problem (Watson 1981), and it would appear that the problem of tracking a free-floating radio pill in order to maintain the correct signal strength had not been completely resolved. A further unsolved problem is the ability to maintain the position of the capsule in a certain area. Most of the studies had been performed with untethered capsules and maintaining it in the stomach was ensured by correct positioning of the patient. This is borne out by a recent study assessing the effect of antacids on gastric pH (O'Sullivan, Harrison, Bullingham 1984). For duodenal measurements, the capsule required to be tethered and control of placement and maintaining this is far more difficult than with pH electrodes attached to naso- or orogastric tubes.

CHAPTER 3

24 HOUR AMBULATORY DUAL GASTRO-DUODENAL pH MONITORING USING A COMPUTER-BASED SYSTEM METHODS: DEVELOPMENT AND VALIDATION

3.1 THE pH MONITORING SYSTEM

The pH monitoring system consisted of two pH glass electrodes which were connected to a portable pH microlog receiving unit. Radiotelemetry techniques were not employed due to the difficulties in placement that have been alluded to in Chapter 2.

3.1.1 THE pH ELECTRODES

The pH electrodes, selected for this investigation, were combined micro pH glass/reference electrodes (GK2801C, Radiometer, Copenhagen, Denmark) (Illustration 3.1). Their small size, being 4.5 mm in diameter and 25 mm in length, made for easy insertion and passage through the pylorus. The electrode consisted of a pH-sensitive glass bulb and, separated by glass fibres as a liquid junction, a built-in silver/silver chloride reference electrode (Figure 3.1). This "built-in" reference electrode obviated the necessity for an external reference lead to the subject's skin, improved the reliability, and minimized electrode drift of the recordings. It also enabled the calibrations to be performed on the laboratory bench without requiring the test subject. A 1M solution of potassium chloride, approximately 30 μ l in volume, saturated the reference electrode.

Each pH electrode was connected to a 1 mm diameter cable, 2.5 m in length, which was covered with a protective silicone tube. This covering served to prevent against external electrical interference as well as cable damage in the patient's mouth.

Changes in temperature affect the pH readings of the electrodes and, for this reason, calibration of the

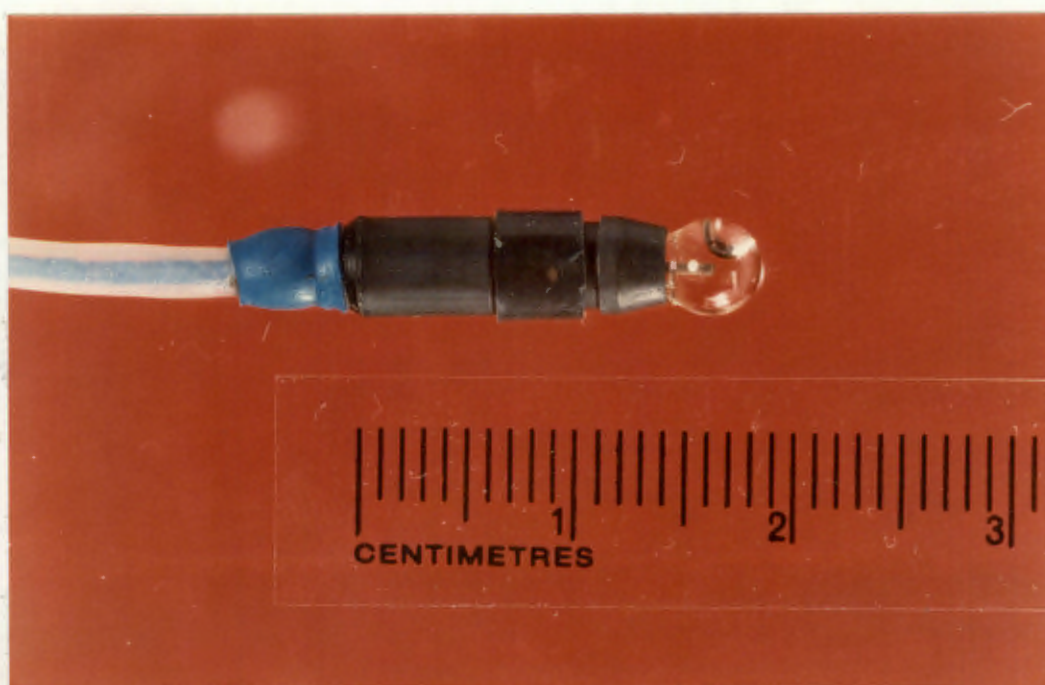


Illustration 3.1 : Combined micro glass/reference electrode

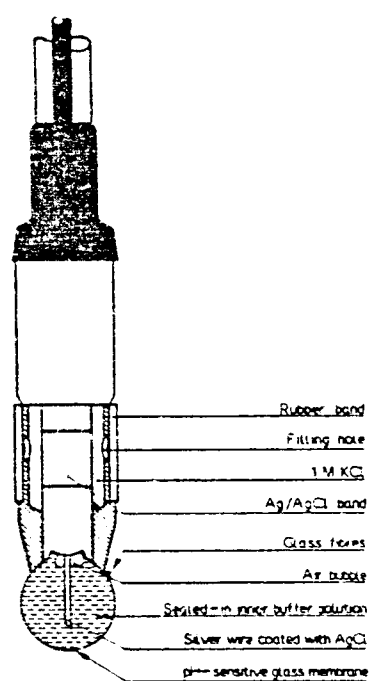


Figure 3.1 : Combined micro pH glass/reference electrode

electrodes before and after each study was performed in buffer solutions equilibrated in a waterbath at 37°C. However, the intraluminal temperature of the gastro-duodenal segment is relatively constant (nocturnal variation <1°C) and the temperature of foodstuffs entering the stomach rapidly equilibrates with body temperature. Thus, minor temperature changes were considered to have a negligible effect on the pH readings. The electrodes were, however, responsive over a temperature range of 0 - 60°C.

Prior to the start of each study, the microlog receiving unit was calibrated by placing the two electrodes in buffer solutions of pH 7.0 and 4.0 (see Section 3.1.2 below). This was repeated at the end of the study so as to check if any drift in the electrode response had occurred. Such drift in the pH reading did sometimes occur, although it was usually very small and did not appreciably alter the recording over the twenty four hour study period. Larger drifts were most commonly due to potassium chloride depletion of the reservoir surrounding the reference electrode. This was avoided by regular weekly flushing and refilling of the reservoir. The other major cause of faulty readings from the electrode was damage to the wires connecting the electrodes to the receiving unit. This was due firstly to the delicate connection between the electrode wires and the female plug which attached to the receiving unit. The wires were of one millimeter diameter which made soldering of the plug difficult. However, stability of the connection was achieved by covering it with heavy duty rubber and looping the wire around the plug. This produced satisfactory results, and, as an added precaution, the study subject was asked to take care of the connection on each occasion. The second cause of damage was due to the patient accidentally biting on the wires whilst eating. This problem was overcome by ensuring the wires

looped lateral to the upper set of molar teeth. In two patients the wires were severely damaged due to grinding of teeth during sleep and this resulted in one set of electrode wires being severed.

3.1.2 THE pH MICROLOG RECEIVING UNIT

The pH microlog receiving unit was designed and manufactured by the Department of Medical Physics at Ninewells Hospital & Medical School, Dundee (Illustration 3.2). It was the latest prototype of recording units modified from an existing system which was originally designed for pH monitoring from a single source. This modification entailed the insertion of dual electrode monitoring facilities. The two input electrodes were referred to as the proximal and distal electrodes. The unit contained an 8-bit CMOS (complementary metal oxide semi-conductor) Motorola microprocessor which co-ordinated and executed all functions. The machine code programme was held in CMOS "read only memory" (ROM). The input from the pH electrodes was an analog signal, the voltage of which was proportional to the measured pH. This voltage was amplified in the receiving unit, filtered then converted to an 8-bit binary value by an analog-to-digital converter (ADC). In order to restrict these digital readings to the working range of the logging unit, the signal was corrected according to the calibration values recorded at the beginning of each study. The pH reading was then entered into the digital memory of the unit. The present unit had a memory capacity of 16 kilo bytes, but due to software limitations, each study in this present work utilised just over half of this memory space.

The timing of the pH sampling was controlled by a calender-clock integrated circuit incorporated into



Illustration 3.2 : pH Microlog receiving unit and 4 button detachable keypad

the unit. This also enabled the current date and time of day to be logged. The power source of the unit was six rechargeable nickel-cadmium batteries (1.2 volt) which provided adequate and steady power for up to five days between charges.

The front of the logging unit had a liquid crystal display screen, which was able to display a message of up to thirty-two characters. On this screen were shown the current time of day, dual mode of sampling, and the voltage from the proximal electrode in digital form with a range of 000 to 239. Next to this screen, was the "event" button. With the receiving unit operating in the logging mode, the "event" button, when pressed by the study subject, started a sequence of four events - a) Had pain, b) Lay down, c) Stood up, d) Ate meal. This allowed the subject to indicate, during the recording, changes in posture and times of meals or drinks. Having pressed the button, the appropriate message was selected by pressing the button a second time. The information was immediately logged into the unit's memory, between data points from the two electrodes. For the present investigation, the subject was instructed to utilise "Lay down" when going to bed at night, and "Stood up" when getting up in the morning. This allowed clear separation of the daytime and nocturnal recordings.

The function mode of the receiving unit was controlled by a detachable four-button keypad (Illustration 3.2), which was used at the start and end of each study. There were five function modes:

a) Idle mode: This was the unit's "off" mode and the mode during which recharging of the batteries was undertaken.

b) Set-up mode: This allowed for the receiving unit to be pre-set according to the parameters of each study. This included the source of input (pH electrode or radiotelemetry capsule), single or

dual sampling, parameters of time (year, month, day, hour, minute), sampling rate (range from every one to fifty nine seconds), and setting of the upper limit of the data boundary.

c) Calibration mode: Both electrodes were first placed in buffer solution of pH 7.0 at 37°C and with the unit switched into the calibration mode with sampling rate at once every ten seconds, calibrating recording commenced. The final (tenth) reading from the proximal electrode was stored in the unit's memory as the calibration value for pH 7.0. The electrodes were then placed in buffer of pH 4.0, and the calibration process continued. Again the final reading was stored as the calibration value for pH 4.0. After this final recording, the unit automatically switched into the idle mode, and was ready for use. For the "end" calibrations at the termination of each study, the electrodes were placed again in the two buffer solutions (first buffer pH 7.0). With the receiving unit switched to the logging mode, further recordings were made for 3-5 minutes in each buffer solution.

d) Logging mode: In this mode, the voltages from the pH electrodes were recorded and stored in digital form in the memory of the receiving unit. Once turned on, the keypad was detached, and reapplied to end the study period to switch the unit into the idle mode.

e) Signal mode: The unit was tuned for each electrode using this mode by adjusting screws on the side of the box. A measure of the signal strength was provided, with voltage readings being displayed at once every second. The voltage of the buffer solution pH 7.0 was near 40, whilst that of buffer pH 4.0 was approximately 120. This mode of function was also used for the accurate placement of the electrodes within the duodenal bulb, in combination with the fluoroscopic screening (see Section 3.3 below).

As the electrodes varied slightly in sensitivity, it was possible that different pH readings from the same pH buffer solutions might be recorded by the receiving unit. To minimize this discrepancy, the unit was tuned for each electrode in turn by using two controls: offset control, which adjusted the absolute voltage recorded for a particular pH; and gain control, which adjusted the voltage change between two pH levels. With the first electrode placed in a buffer solution of pH 7.0, the voltage reading from the electrode was corrected by adjusting the offset control. The voltage was then read with the same electrode placed in buffer of pH 4.0, and the unit was calibrated to the correct rise in response using the gain control. The procedure was repeated with the second electrode. This was done as accurately as possible to ensure the same voltage readings from each electrode in the same buffer solutions. To reduce to a minimum the extraneous interference in the electrode reading, the unit had a built-in filter with a time constant of less than one second. This smoothed the signal and produced a step response of approximately 80% in one second.

The size of the unit was 19 x 13 x 4.5 cm and, weighing only 720 grams, it was readily transportable. The unit was placed in a sleeve which enabled it to be worn on a belt around the subject's waist for the duration of the study. This ensured freedom of movement and, with the electrodes in situ, the subject was able to resume normal daily activities.

The sampling rate of the unit could be selected from a range of 1 to 59 seconds. For this present study, the rate was set at once every 20 seconds. The software limited the upper boundary of memory to 8200 data points which, over a 24 hour period, allowed the fastest sampling rate for dual monitoring to be every 20 seconds.

The main problem encountered with the receiving unit was one of data loss. Fortunately, this rarely occurred. The first instance when this might occur is when the batteries lose charge. To prevent this, the units were recharged twice weekly, but due to the normal lifespan of the batteries, loss of charge can occur rapidly with older batteries. If for some reason, the computer is unavailable for data transference and storage, the receiving unit should be connected to the recharging circuit to guarantee against loss of battery charge and hence data loss.

The second cause of data loss occurs when a new calibration sequence is started accidentally before the recorded data in the unit's memory has been transferred to the computer for storage. Initiating the calibration mode automatically resets the unit's memory counter to zero. If this occurs, the set-up mode is entered, and the memory boundary is brought forward to its original point. The recorded data can then be transferred to the computer for permanent storage.

3.2 RESPONSES OF pH MONITORING SYSTEM

The response of the electrodes in buffer solutions of pH 1.0 through to pH 8.0 at 37°C was investigated in vitro. Beakers with buffer solutions of pH 1.0, 2.0 to 8.0 (pH Buffers, Russell pH Ltd., Fife, Scotland) were placed in a water bath at 37°C and allowed to equilibrate to temperature. The two electrodes then were placed sequentially in each of the buffer solutions for a period of 30 minutes and the pH recorded. The response in these solutions is shown in Figure 3.2, and is a linear one.

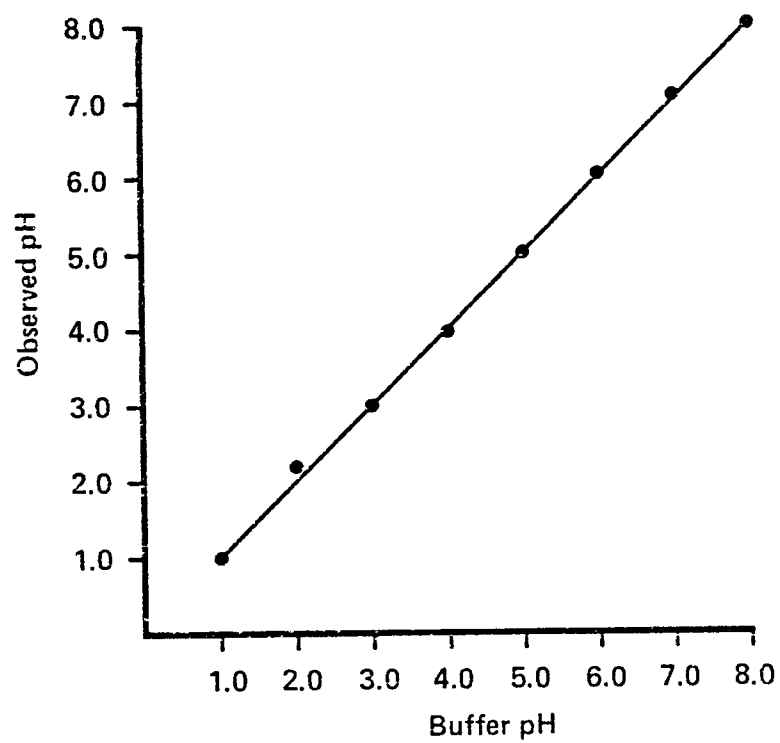


Figure 3.2 : Response of the electrodes in buffer solutions

The response of the electrodes and receiving unit together was measured by placing one electrode in buffer pH 7.0 and rapidly plunging it into buffer pH 4.0 then back into buffer pH 7.0. The sampling rate of the receiving unit was set at one per second. The step response was exponential and almost symmetrical for the rises and falls, measuring 90% in one second. Similar responses were obtained with the second electrode.

3.3 In Situ PLACEMENT OF THE ELECTRODES

Two pH electrodes were used simultaneously, and they were strapped together such that their glass bulbs were 9 cm apart. Initially, black silk sutures were used to tie the electrodes and their leads together but this caused irritation in the subject's pharynx on swallowing. The sutures were replaced by small rubber bands (originally designed to cover the refilling hole over the KCl reservoir on glass electrode) and this made the assembly infinitely more comfortable.

A small rubber bag, filled with 14 gm of mercury, was attached to the distal electrode by means of a 20 cm length of fine PVC tubing (Portex, London) (Illustration 3.3). The bag was made from three finger tips of a surgeon's glove; each being individually tied to prevent leakage. At the beginning of the study, the bag was dipped into molten latex rubber in order to secure the tear-drop shape. This process was later not found to be necessary as it did not provide any advantages. The size of each bag, approximately 3.5 cm in length and 8 mm in diameter, was of great importance in order to negotiate passage through the pylorus, especially in duodenal ulcer patients.

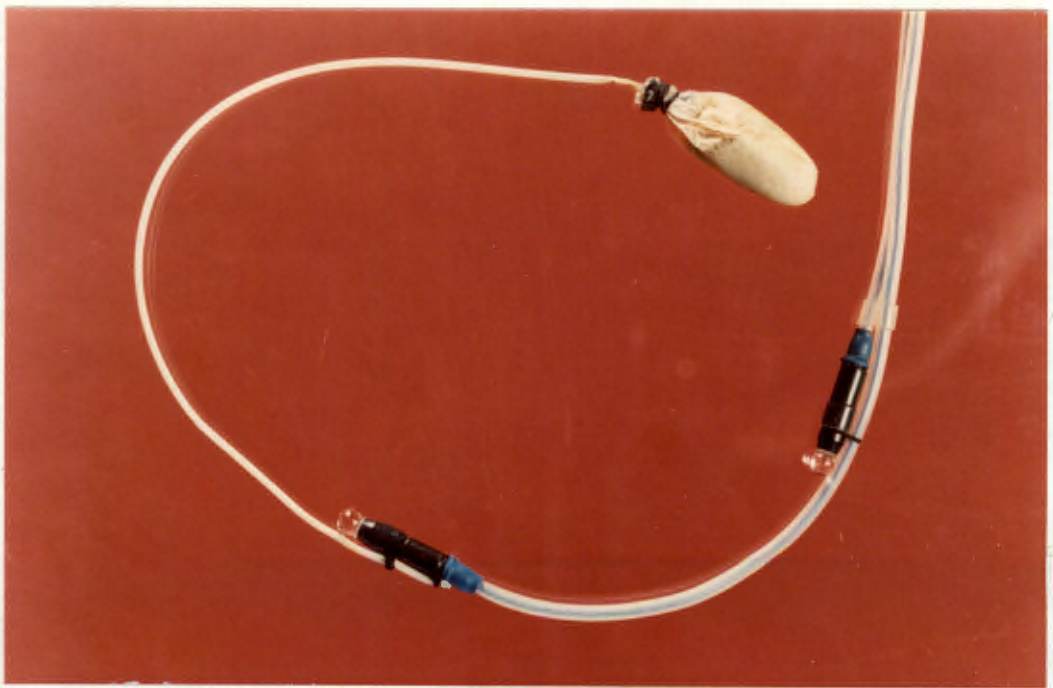


Illustration 3.3 : pH Monitoring system consisting of the two pH electrodes strapped together and the weighted bag

After an overnight fast, the subject's pharynx was sprayed with Xylocaine^R Spray (Astra Pharmaceuticals Ltd., Kings Langley). The weighted bag and pH electrodes were passed orally and swallowed by the subject. Approximately 70 cm of electrode lead was then introduced into the stomach. The subject was instructed to lie on his right side, so as to position the bag in the antrum. After 15-20 minutes, the receiving unit was switched to the signal mode and the voltages from the two electrodes were read on the liquid crystal screen. The displayed voltages indicated the positions of the electrodes in terms of the pH. As the distal electrode was pulled through the pylorus into the duodenum by the weighted bag, the voltage changed abruptly from approximately 190-210 to 70-80. The subject was then instructed to sit up on two pillows while the weighted bag was pulled more caudally into the jejunum. The exact positions of the electrodes were then obtained by lying the subject flat. Using a combination of fluoroscopic screening and the digital read-outs from the receiving unit, the distal electrode was positioned in the apex of the duodenal bulb whilst the proximal electrode lay along the lesser curve of the antrum. The weighted bag came to lie in the region of the duodeno-jejunal flexure (Figure 3.3). The excess length of electrode leads was withdrawn without compromising the positions of the electrodes, and were attached to the subject's cheek with adhesive tape. The positions of the electrodes were maintained by the caudal peristaltic pull on the weighted bag against fixation of the leads at the subject's cheek.

This method of maintaining the positions of the electrodes within the gastro-duodenal segment using a weighted bag has previously been shown to be reliable in producing a stable position (Eyerly 1942, McCloy, Vickery and Baron 1980). These findings were confirmed in the present study. At the end of each study period,

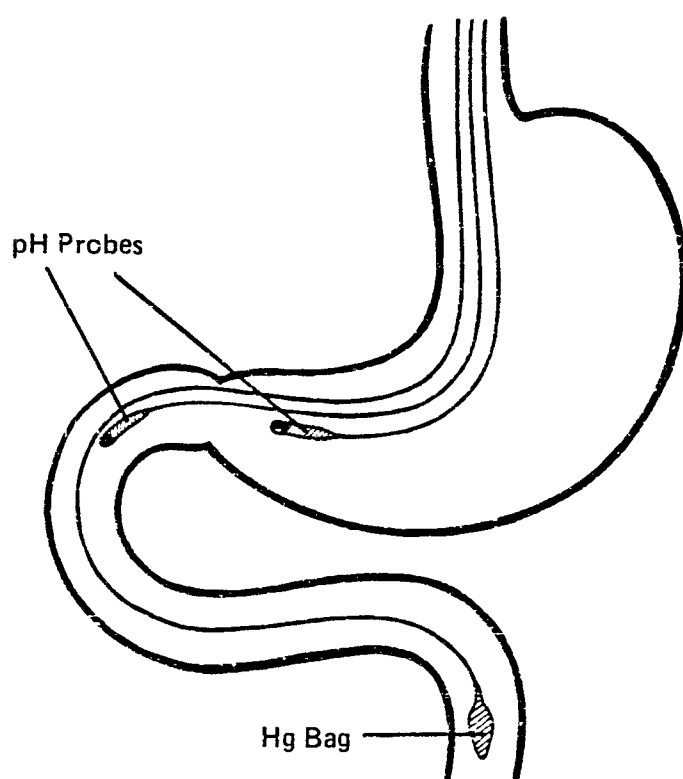


Figure 3.3 : The monitoring positions of the two electrodes within the stomach and duodenum

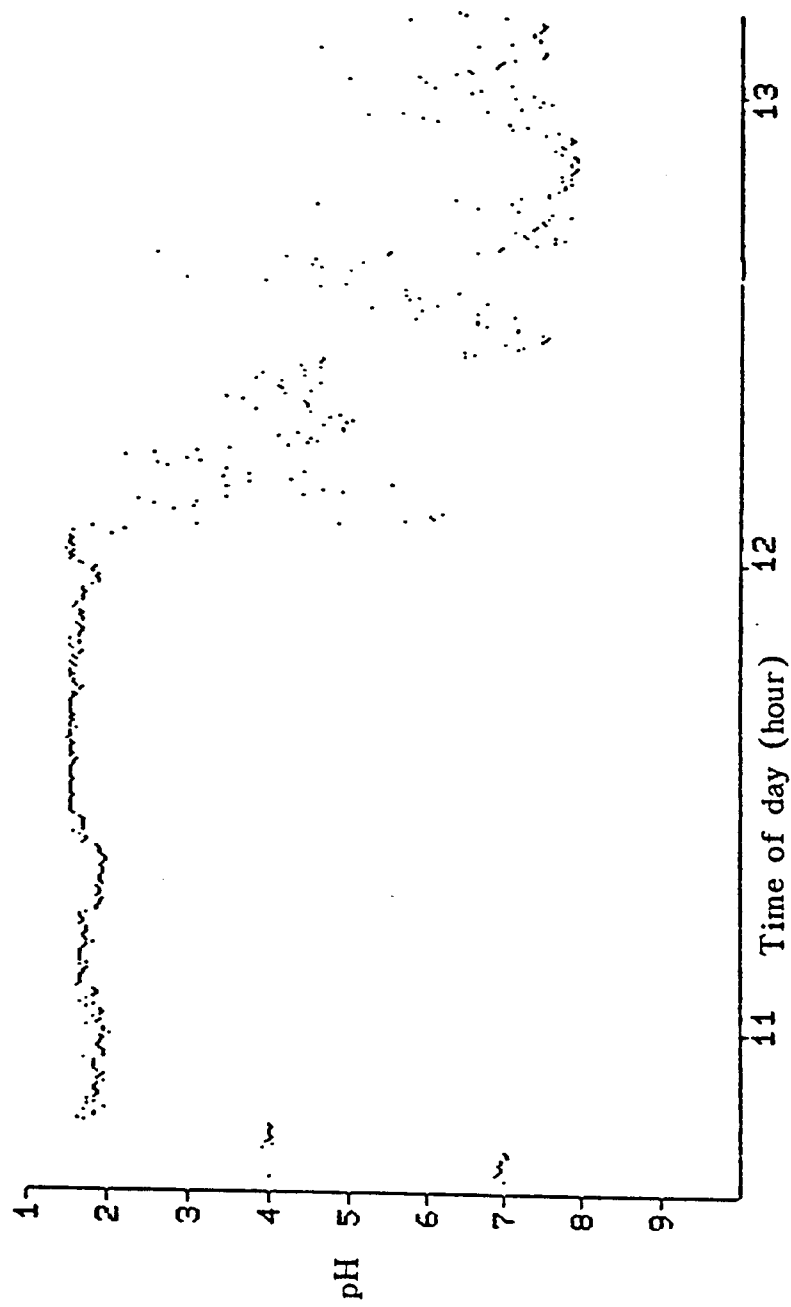


Figure 3.4 : Change in pH values measured by the electrode passing from the antrum (left) into the duodenum (right)

the positions of the two electrodes were checked with fluoroscopic screening and in only five out of the forty eight studies performed was a shift in position encountered. One patient, during a coughing fit, vomited both electrodes into the oesophagus. After a few swallows of water and a short interval, the electrodes returned to the previous positions in the duodenum and antrum and this was confirmed by the pH tracing. The abrupt change in pH between the antrum and duodenal bulb can readily be seen in Figure 3.4.

3.4 COMPUTER OPERATING SYSTEM

The use of computers in the field of medical research has rapidly expanded over the last few years. The enormous amount of raw data collected in modern investigations, while occupying a large storage space, make analyses by hand tedious, time-consuming and possibly open to human error. The often repetitive and meticulous data analyses are far better suited to computers and the results are rapidly obtainable. The system also allows for reliable reproducibility of the results.

The present investigation utilised the existing computer hardware (an IBM PC computer, 256K of memory), of the University Department of Surgery, for the storage and retrieval of the 24 hour pH data. Hard copies of the results were obtained by an Epson RX-80 dot matrix printer connected to the IBM PC. These were shown both as graphic and numerical forms. Two options of graphics were available: a longitudinal plot of the 24 hour data, and a copy of the screen display.

3.4.1 DATA STORAGE

Two methods of data storage were used: the hard disc, which has a very large storage capacity; and the floppy disc, which has a small limited capacity. The collected pH data was off-loaded from the receiving unit to the IBM-PC computer and stored on hard disk. The hard disc was of the Winchester type (sealed units spinning continuously), and part of the Medical School's "CORVUS OMNINET" network. Each disc had eighteen mega bytes of storage space which was shared with other departments of the Medical School. The advantage of the hard disc, apart from the increased capacity, was that it allowed rapid access to the data, as well as access to other software programmes on the network for various analyses. Towards the latter part of the study, the computer network was refurbished, and subsequent pH studies were off-loaded from the receiving unit directly to 5.25 inch floppy discs for storage. Each floppy disc had storage capacity for approximately 36 studies (each having 8200 readings). Data stored on hard disc was later copied to floppy discs and all analyses were done using these discs. Backup copies of the data from both storage units were made on to floppy discs and kept separately. This is a vital part of any computer system as accidental loss or data corruption can occur.

3.4.2 COMPUTER SOFTWARE

The computer programme (Department of Medical Computing, Ninewells Hospital & Medical School, Dundee, Scotland) utilized the "p-" operating system and PASCAL programming language. The programme was divided, via the main menu, into three main components; these being the filing system, the graphics system and the

analysis system. As each system was entered, a number of options were available, each of which branched out into further sub-divisions.

The filing system allowed for data storage together with manually entered subject and record details of name, date of birth etc. Each pH reading was stored in digital form, 001 to 239, with the events being entered in a similar fashion, 240 to 255. Correction or alteration of the events was possible and was used both for correction of the study subject's mistakes (event omission or wrong event) and for segmental analysis of the record (see below). The raw pH data were in no way able to be altered with this programme. However, drift in the electrode response can occur and can be significant over prolonged periods. A correction programme was available that assumed the drift was linear, and each data point was altered accordingly. In practice, the drift in response that infrequently occurred was minimal and did not require correction by the software. As for the receiving unit, the programme was initially written for analysis of pH data from a single source, and this was modified for the present study to accommodate the dual pH monitoring system. It was set such that alternate data points were analysed together: the first, third, fifth etc. data points were obtained from the proximal (gastric) electrode, and the second, fourth, sixth etc. data points were from the duodenal electrode.

The graphics system allowed for the data to be either displayed on the screen (Illustration 3.4), or plotted by the printer. The screen was able to display approximately 300 data points at one time. The current and elapsed times were shown in the corners of the screen. Sequential segments of the recording could be displayed by moving along the tracing, and a cursor

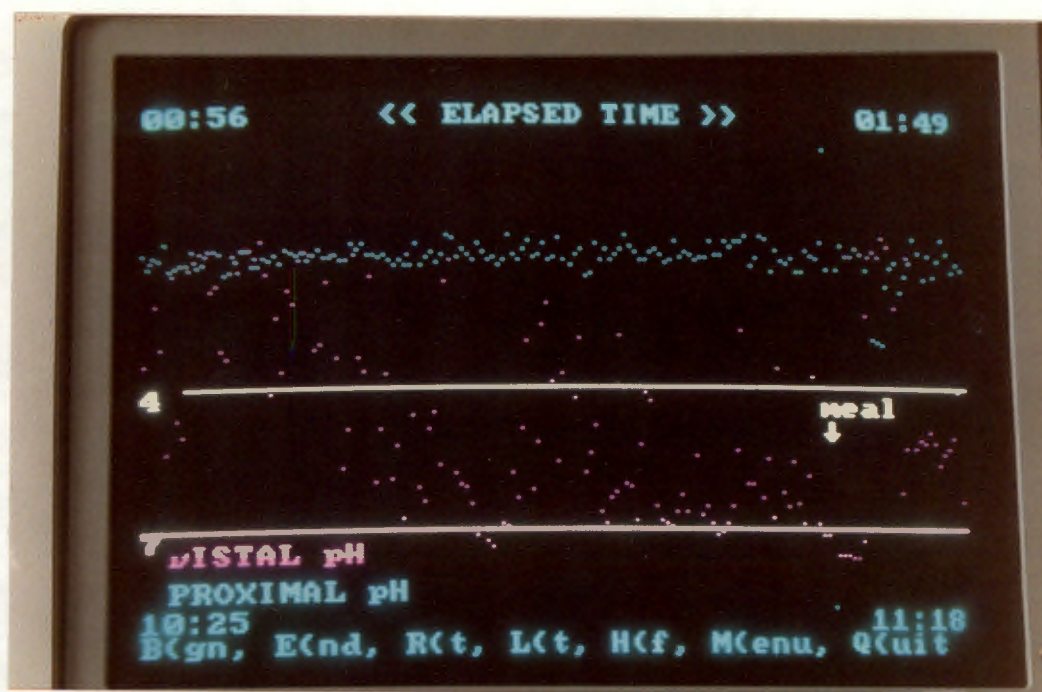


Illustration 3.4 : Graphics display of part of the 24 hour

pH data as shown on the computer screen.

The duodenal pH data (distal) is shown by the pink dots, and the gastric pH data (proximal) by the blue dots. The time of day is shown along the X axis, with the start and finish times in the bottom corners of the screen. The pH is shown on the Y axis, with pH 1.0 at the top. The time of a meal is illustrated

facility was present to accurately identify times of particular events. The profile of the full 24 hour record was plotted on a longitudinal graph. The pH scale 1 - 9 was shown vertically, and the actual time, in hours, was plotted on the X axis (Figure 3.5). Each pH reading was plotted as a single dot, and the programme allowed for either the proximal, distal or combined records to be plotted. The events were also displayed at the appropriate times. The second graphics display was that of the frequency distribution of the pH readings. The software sorted the readings according to their digital incidence, i.e. 1 - 239. The corresponding pH value was calculated from the calibration values and the distribution as a frequency histogram was then plotted (Figure 3.6).

3.4.3 ANALYSIS OF pH DATA

The data was analysed in two main sections: firstly, to determine the overall acid exposure, and secondly, to examine the pattern of this exposure. In both analyses, the daytime and nocturnal data were considered separately, and the results were available either on the screen or as a printed hardcopy.

1. Cumulative Percentage of pH Exposure

This analysis was used to determine the pH profile as a frequency distribution of the data. For the duodenal bulb pH, the incidence of recordings from the duodenal electrode was calculated for each pH level over the full pH range, and the exposure was expressed as the cumulative percentage of the total pH readings. Thus, the percentage of time that the pH of the duodenal bulb was below levels pH 9 to pH 1 was reported on. The results were represented in graphic and numerical format. For the graphics, the plot is in the form of a frequency histogram with the range of pH levels shown along the X

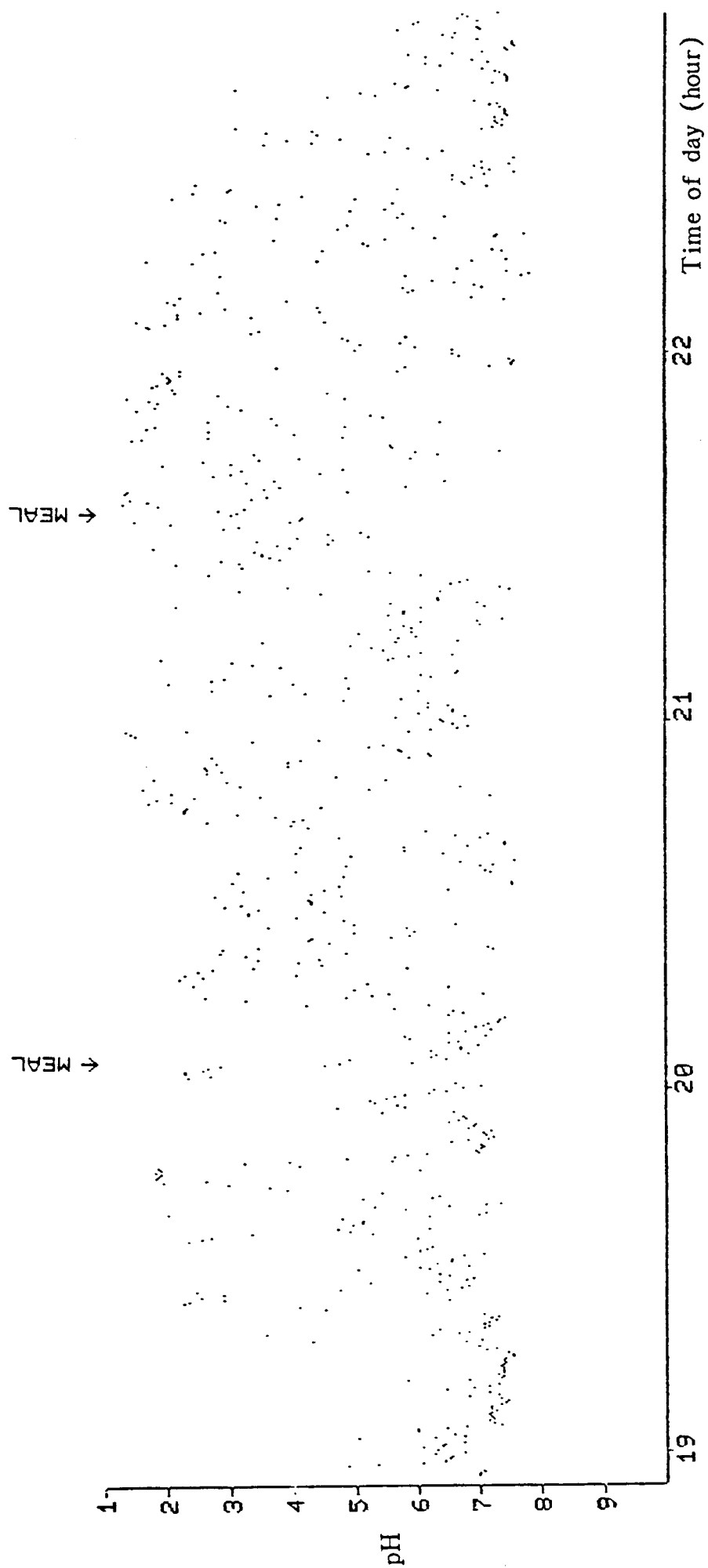
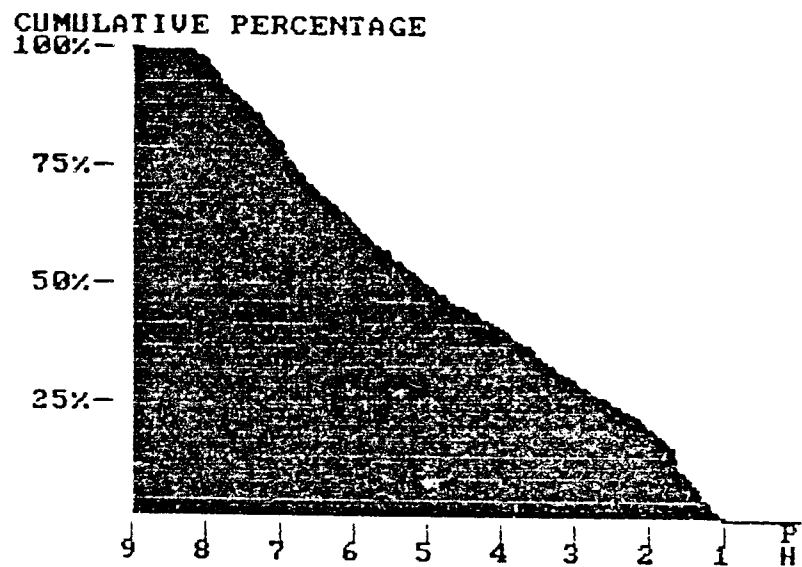
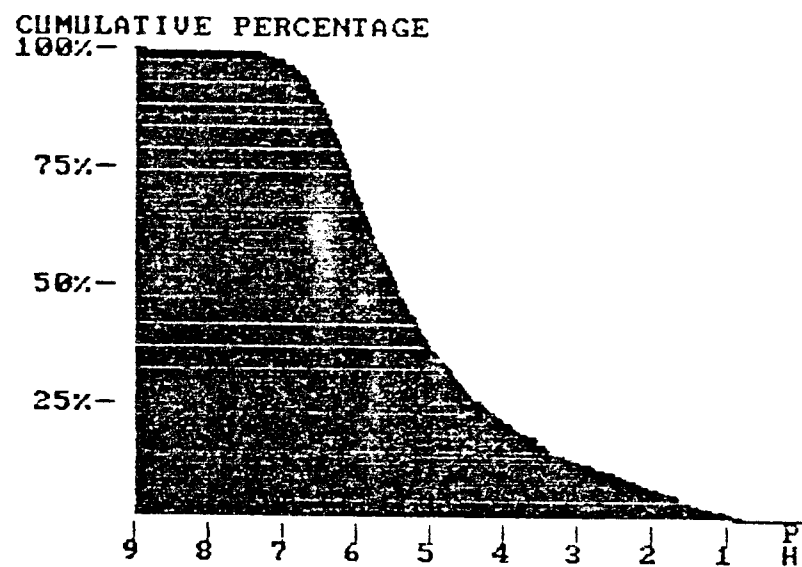


Figure 3.5 : Section of the duodenal bulb pH tracing, showing the rapid fluctuations in pH and the acidification following meals



PERCENTAGE OF DATA BELOW PH VALUE

	pH 9	8	7	6	5	4	3	2	1
RECORD: 768									
ERECT	100.0	100.0	97.8	68.6	36.6	21.4	12.4	7.0	1.5
SUPINE	100.0	97.8	80.1	62.5	49.9	40.8	29.5	19.8	0.7
TOTAL	100.0	99.2	91.1	66.3	41.6	29.8	18.9	11.9	1.2

Figure 3.6 : Frequency distribution of the total 24 hour duodenal pH data, showing separately the daytime (above) and nocturnal (below) recordings

axis, and the cumulative percentage of total data points on the Y axis (Figure 3.6). The daytime (erect) and nocturnal (supine) exposure are reported separately. The exact percentages are shown numerically under the graphics display.

For the gastric pH data, the computer calculated the frequency distribution as described above. This gave figures for distribution below particular pH levels. Thus, by subtracting these computed results from 100, the frequency distribution of the data to above particular pH levels could be obtained.

2. Analysis of Acid Peaks within the Duodenal Bulb

An acid peak was defined as two or more consecutive pH readings below a particular "baseline" pH level; that is, with a pH sampling rate of once every twenty seconds, the minimum time for such a peak was 40 seconds. The end of each acid peak was determined by the following two consecutive readings above the same pH level. The computer programme allowed for the baseline pH level for this analysis to be selected, thus enabling acid peaks to below pH 5 through to pH 1 to be determined. The acid peaks were considered to be of short or long duration, and at the latter stages of this study, the computer software was modified to enable the time limit of such acid peaks to be pre-selected from a range of one to five minutes. Thus, with a time limit of 4 minutes, short-lived peaks were those peaks less than four minutes duration, whilst long-lived peaks were of greater than four minutes.

The results were displayed either in detailed (Figure 3.7) or summarised form (Figure 3.8). For the latter, the data was standardised to one hour. Five parameters were investigated:

	REFLUX EVENT #	24 H TIME	REFLUX POINTS	MIN.	TOTAL POINTS	TOTAL MIN.	EVENT
12:01 - 13:30							
	1	12:06	2	0.7	2	0.7	
	2	12:17	2	0.7	4	1.3	
	3	12:18	3	1.0	7	2.3	
	4	12:22	6	2.0	13	4.3	
		12:28					MEAL
	5	13:00	6	2.0	19	6.3	
	6	13:03	10	3.3	29	9.7	
	7	13:09	25	8.3	54	18.0	
	8	13:18	3	1.0	57	19.0	
	9	13:20	2	0.7	59	19.7	
	10	13:23	26	8.7	85	28.3	

(REFLUX DETECTED WHEN PH<4)
ERECT

	<u>DURING STUDY 1.51 H</u>	<u>STANDARDISED 1 H</u>
POINTS	95	
TOTAL DURATION	31.67 MINS	21.03 MINS
REFLUX POINTS	85	
TOTAL DURATION	29.33 MINS	18.82 MINS
REFLUX EVENTS	10	6.64
AVERAGE DURATION	2.83 MINS	2.83 MINS
LOST POINTS	0 0.0%	

DISTRIBUTION OF REFLUX EVENTS

< 5 MIN : 5.31 Events per hour
=> 5 MIN : 1.33 Events per hour

Figure 3.7 : Detailed analysis of acid peaks within the duodenal bulb to pH <4

	RECORD NO.	TIME pH <4 (min)	NO. OF EVENTS	DURATION PER EVENT (min)	REFLUX PATTERN(no. of events)	
					< 5 MINS	> 5 MINS
All data standardised for one hour	768				-----	
	ERECT	11.16	3.50	3.19	2.70	0.90
	SUPINE	23.00	6.58	3.50	5.14	1.44
	TOTAL	15.65	4.67	3.35	3.63	1.04

Figure 3.8 : Summarised analysis of acid peaks within the duodenal bulb to pH <4

- a) Time spent below below a the pre-selected pH level (minutes per hour)
- b) The number of acid peaks per hour
- c) The mean duration of the acid peaks (minutes per hour)
- d) The number of short-lived acid peaks (no. per hour)
- e) The number of long-lived acid peaks (no. per hour)

3. Analysis of pH rises within the stomach

The rises in pH within the stomach were analysed using the programme described above, with one important alteration. The direction of pH testing was changed from "lower than" to "higher than" the pH level. As with the acid peaks, a rise in pH was defined as two or more consecutive data points above a pre-selected pH level, and ceased when two consecutive points subsequently fell below the same pH level. The paramters for analysis were similar.

3.5 STATISTICAL ANALYSES

The results of data stored on the hard disc were transcribed on to floppy discs, and the statistical analyses for all the pH records were performed using these discs. The results of the pH measurements were not normally distributed, making the use of means and standard deviations inappropriate. For this reason, median values were selected for analytical purposes. The data recorded during the day was compared with that of the night using the Wilcoxon Matched-Pairs Signed-Ranks test for non-parametric data. Having studied a cohort of healthy subjects to obtain the normal pH profiles, a group of patients with active duodenal ulcer were studied. Their results were compared to those of the controls, and the analysis performed using the Mann Whitney U test.

3.6 IN SITU USE OF THE DUAL GASTRO-DUODENAL pH MONITORING SYSTEM

Intubation of the pylorus was the first difficulty encountered during placement of the dual electrode system. The normal healthy pylorus allowed for easy passage of the weighted bag, and this was borne out by the author who swallowed the system on four separate occasions. The mean time for this was approximately 35 minutes. Patients with active duodenal ulcers invariably had a degree of oedema of the pylorus which caused some obstruction to the passage of the weighted bag. This problem was noted many years ago during studies with aspiration catheters (Morton 1929). The size of the bag was thus most important, and three variations were constructed until a satisfactory size and weight combination were obtained.

Limited fluoroscopic screening was used to aid in the correct placement of the distal (duodenal) electrode and used a second time at the end of the study period to re-check the positions. An attempt was made to position the electrode using only the digital read-outs of voltages displayed on the receiving unit, but this proved inaccurate and unreliable in obtaining reproducible results. Previous investigations have also employed fluoroscopic techniques (Tomenius and Williams 1960, McCloy, Vickery and Baron 1980, Hannibal and Rune 1983), some included the use of contrast media to outline the duodenal cap (Bircher et al 1965, Rhodes and Prestwich 1966). Repeated use of fluoroscopic screening is inappropriate in long-term studies. Measurement of the potential difference across the pylorus has been used to indicate the electrode remaining in the bulb (Andersson and Grossman 1965, Archambault, Rovelstad and Carlson 1967, Rune and Viskum 1969). A

more elaborate method of placement has been described using a "chain" of four electrodes strapped very closely together (Rune 1981). Examination of the tracings enabled the exact positions of each electrode to be determined. However, this would not be entirely suitable for long-term pH monitoring as the presence of four electrode leads in the subject's pharynx would help with compliance.

There was a learning curve for the correct placement of the electrodes using the method described above. The mean time required for this during the complete study was 50 minutes, but ranged from 30 to 100 minutes. The initial problem encountered was that the distal (duodenal) electrode prolapsed back into the stomach, and this occurred in four studies. These cases occurred early in the investigation, and on examining the pH tracings, it was evident that, as the subject sat up, the distal electrode prolapsed into the stomach with not enough length of lead to enable it to pass back into the duodenal bulb. A further problem was encountered in a single patient in whom excess length of lead was placed in the stomach due to difficulty in intubating the duodenum. This resulted in both electrodes being pulled into the duodenum by the peristaltic action on the weighted bag.

Guards or shields around the electrode bulbs were not used in this study. Suspicion that the high pH values recorded in the duodenum were artefactual was increased by reports of widely differing pH readings of the luminal contents and the duodenal mucosa (Bircher et al 1965). However, as these shields were found to be clogged with mucus at the end of the recordings, it was felt that the presence of a shield "softened" the pH curve and were more likely to produce artefactual readings than bare electrodes (Rune 1981). Evidence against artefactual readings being caused by mucosal contact can be seen in

the steady recordings made within the antrum. Contact with the mucosa is as likely to occur as it is in the duodenum. Thus, the contact does not influence the pH reading of the overlying fluid, and the measured pH will be an accurate reading of the acidity to which the mucosa is exposed.

There has been much debate whether the acidity should be analysed in terms of pH or H^+ ion activity (Bircher et al 1965, Moore and Scarlata 1965, Lucas 1977, Rune 1981). H^+ ion activity is not quite the same as H^+ ion concentration (Walt 1986) and requires an activity coefficient (Moore and Scarlata 1965). Conversion of pH to H^+ ion concentration can be obtained by using Moore's table (Moore 1968). As H^+ ion concentration is minimal at a level of below pH 5.0 (Walt 1986), duodenal acidity (higher pH) is better suited to pH measurements (Mitchell, Hunt and Grossman 1962). Either method may be used for gastric acidity measurements, but comparisons here must be made only between the same units (Walt 1986). For this present study, measurements were made in pH units and not converted to H^+ ion activity or concentration.

The sampling rate of pH measurements was set at once every twenty seconds. As already mentioned, this was the fastest rate allowed with the dual monitoring system over a full 24 hour time period, due to software limitations. Extremely brief pH fluctuations have previously been noted in the duodenum (McCloy, Vickery and Baron 1980, Rune 1981). But the importance of these rapid peaks in the pathophysiology of duodenal ulcer disease is unknown. Due to their extremely short duration, their influence in the acid exposure over a 24 hour period is questionable. These rapid fluctuations were also noted in this investigation when the pH was monitored in the fasting state with the

sampling rate increased to once every second. The tracing of the duodenal pH from this subject is shown in Figure 3.9.

Figure 3.10 shows the duodenal pH tracing of a study performed during the setting up period, with the sampling rate at 20 second^{-1} . The fluctuations in pH prior to the meal (a fasting period) can be seen. The meal consisted of a 150 ml. drink of meat extract and the subsequent rise in duodenal acidity is readily evident. The concurrent gastric pH tracing is shown in Figure 3.11 which demonstrates the rise in pH soon after the ingestion of the meal. This is followed by a return to the pre-meal steady state. The frequency distribution plot of the duodenal pH data illustrated in Figure 3.10 is shown in Figure 3.12.

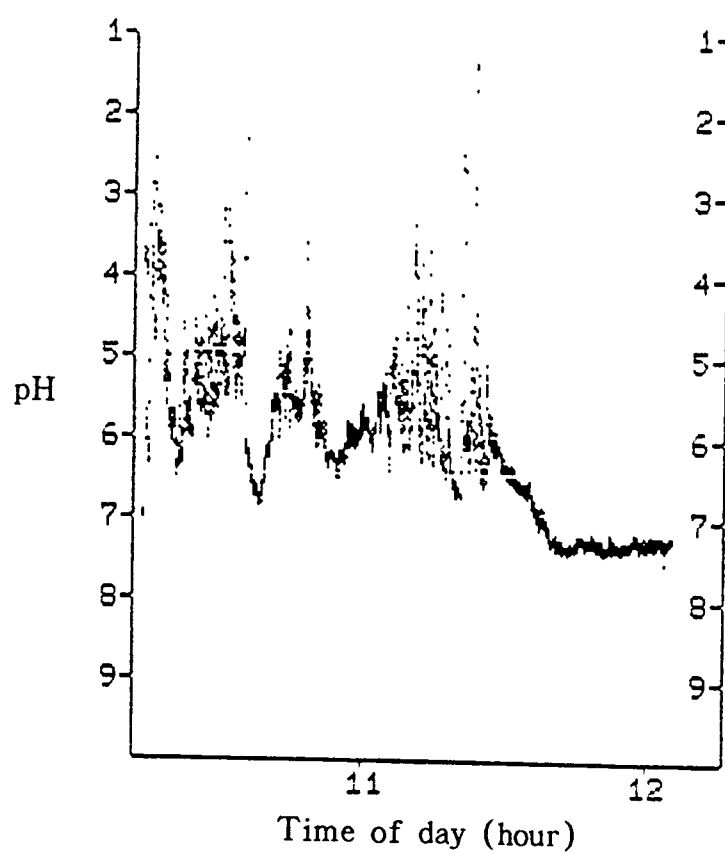
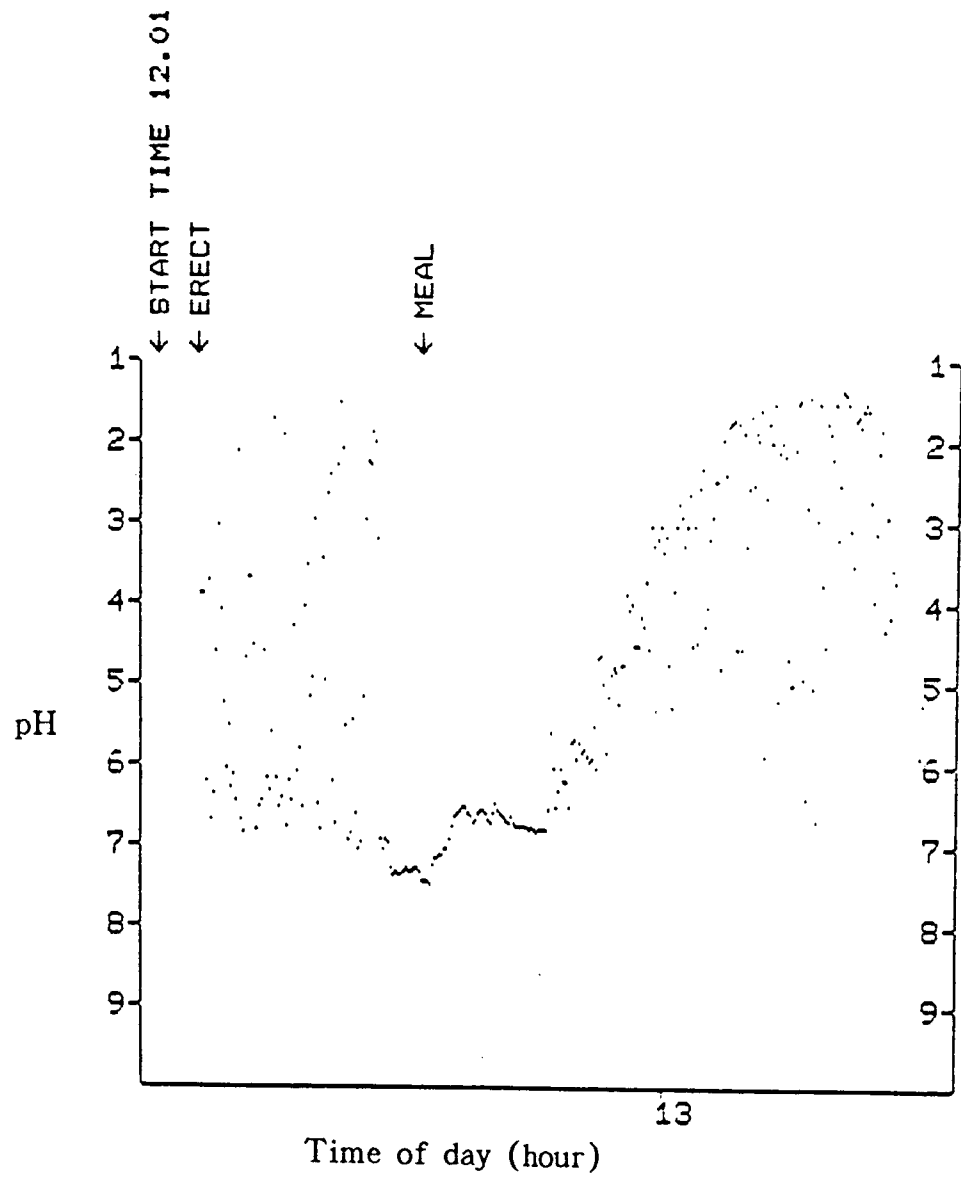


Figure 3.9 : Tracing of fasting duodenal bulb pH, with the sampling rate at 1 sec^{-1}



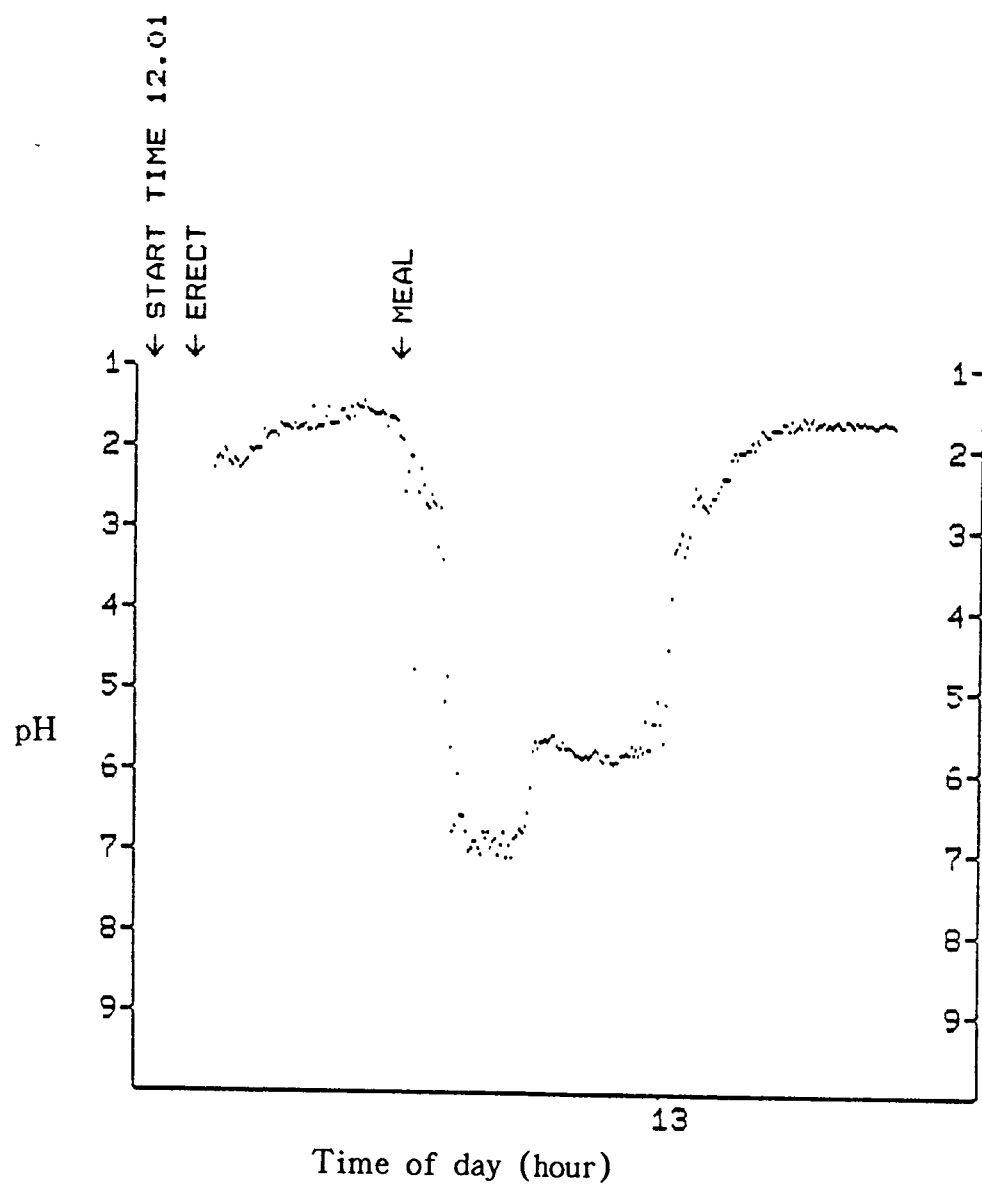


Figure 3.11 : Tracing of antral pH during the same fasting and post-prandial periods as shown in Figure 3.10

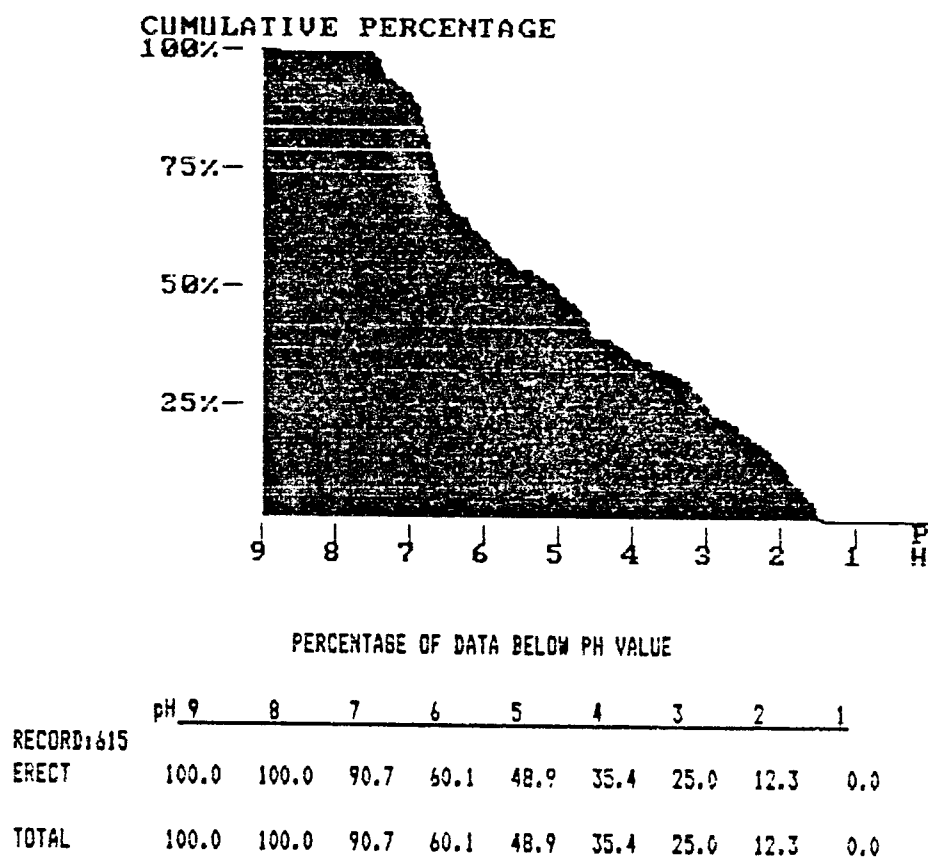


Figure 3.12 : Frequency distribution of the duodenal pH data illustrated in Figure 3.10

CHAPTER 4

THE NORMAL PROFILE OF 24 HOUR AMBULATORY DUAL GASTRO-DUODENAL pH IN HEALTHY SUBJECTS

4.1 STUDY AIM

As discussed earlier in Chapter 2, studies concerning the role of gastric acid in the pathogenesis of duodenal ulcer disease have largely concentrated on the pathophysiological abnormalities of gastric acid secretion (Winkelstein 1935, Wormsley and Grossman 1965, Lam and Sircus 1975, Malagelada et al 1977, Feldman, Richardson and Fordtran 1980). Using these results, the situation occurring in the duodenal bulb has only been inferred. Following the development of new materials and techniques, more recent studies regarding the exposure of the duodenum to gastric acid have been performed (McCloy, Vickery and Baron 1980, Bendtsen et al 1986). However, there are several limiting factors of these studies: a) for the most part, they have been short-term (3 - 5 hours duration); b) not all the "normals" have been free from gastro-intestinal disease; c) the studies have not truly reflected the normal situation in that they have not been ambulatory, and the subjects were studied in a somewhat artificial environment.

The present investigation sought to avoid these shortcomings. The study was set out to achieve three main aims:

1. To record, for the first time, a continuous, ambulatory, twenty four hour, simultaneous profile of gastric and duodenal bulb pH in normal healthy subjects. The normal fluctuations in the daytime and nocturnal gastric acid secretion would be reflected in changes in the gastric pH, whilst the changes in the exposure of the duodenal bulb to such acid secretion would also be recorded.

2. To record the influence that the cephalic phase of gastric secretion might have on the respective gastric and duodenal bulb pH.

3. To record the respective gastric and duodenal bulb pH changes in response to the gastric phase of gastric secretion.

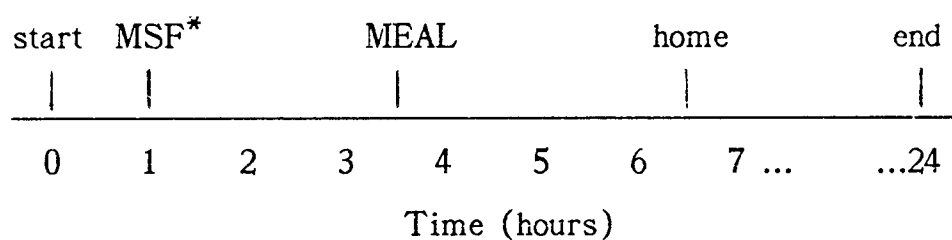
4.2 STUDY GROUP

Nineteen normal healthy subjects were studied. This group comprised healthy volunteers who had no symptoms, nor previous history, of oesophageal, gastric, duodenal, pancreatic or other gastro-intestinal disease. There were thirteen men and six women, and the mean age was 28.7 years. The ages ranged from 19 to 79 years, and although an even distribution through the ages was strived for, the younger volunteers were more agreeable to undergo the study. Volunteers under the age of 18 years were not accepted for the study.

4.3 STUDY DESIGN - STUDY I (Figure 4.1)

The two pH electrodes in tandem were first calibrated in the laboratory in the buffer solutions of pH 7.0 and 4.0. After an overnight fast, the dual gastro-duodenal pH monitoring system was swallowed by the subject and positioned as described in Section 3.4. With the electrodes in position, the wires were taped to the subject's cheek and connected to the receiving unit. The unit was switched on to the logging mode and the pH recording in the fasting basal state commenced. The subject was allowed up to walk about.

Figure 4.1: Study design - Study I



(*MSF: modified sham feeding)

After one hour of recording in the fasting state, cephalic stimulation by modified sham feeding was performed in order to demonstrate the effect of the cephalic phase of gastric secretion on the intraluminal pH. A standardised solid meal was served (Illustration 4.1), and this consisted of two freshly cooked bacon rolls, one slice of buttered toast and 160 ml of warm Oxo drink (Carbohydrate 54.3 g, Protein 37.5 g, Fat 52.8 g, 3456 kJ). The subject was informed in detail as to the requirements of the test and was instructed to chew the food and then spit it out, making sure none was swallowed. The Oxo drink was similarly allowed to be tasted but not swallowed. The duration of the test was thirty minutes; thereafter the subject was kept fasted until the second part of the test.

Two and a half hours later, a second standardised solid meal (Illustration 4.2) was served and was eaten normally. This allowed the effect of the gastric phase of gastric secretion to be monitored. The meal consisted of 120 g minced meat, 120 g potatoes, 60 g peas, 180 g custard and peaches and 160 ml tea (Carbohydrate 55.7 g, Protein 38.1 g, Fat 23.9 g, 2477 kJ). Following the meal, the subject was not allowed to eat or drink for the following three hours.



Illustration 4.1 : Standardized meal served for Modified
Sham Feeding



Illustration 4.2 : Standardized solid meal

After the three hour post-prandial period, there were no restrictions on eating or drinking for the remainder of the 24 hour study period. The subject was allowed home, with the pH monitoring system in situ and encouraged to resume normal daily activities. Instructions were given regarding the use of the "event" button so that on-line recordings could be made of the times of eating and drinking (meal), going to bed (supine) and rising in the morning (erect). In order to check possible mistakes in using the "event" button, the subject was also asked to write the events on a diary sheet.

At the end of the study period, the subject returned to the hospital, and the positions of the two electrodes were checked using fluoroscopic screening. The keypad was reattached, and the receiving unit switched to the idle mode. The two pH electrodes and weighted bag were withdrawn, and returned to the laboratory for recalibration ("end" calibration), (see Section 3.1.2). The pH data stored in the receiving unit was then transferred to the computer for permanent storage and result analysis.

4.4 ANALYSIS AND STATISTICS

The recorded pH data was analysed using the computer software as described in Section 3.4.3. The statistical methods were those outlined in Section 3.5.

4.5 ETHICAL APPROVAL

The protocol of this investigation was approved by the Ethics Committee of the University of Dundee, and the Tayside Health Board.

4.6 NORMAL DUODENAL BULB pH PROFILE

4.6.1 Fasting duodenal bulb pH

A representative tracing of the fasting duodenal bulb pH is illustrated in Figure 4.2. The rapid fluctuations in pH from the baseline level were seen in all the subjects, although the median values tended to be in the pH range of 5.0 to 7.0. These fluctuations were also noted in the later part of the study and were more pronounced in the immediate post-prandial period.

The fasting period was during the first hour of the study, and the fasting pH was determined by the mode of the pH data recorded during this interval. Results showed the mean fasting duodenal bulb pH to be pH 6.73, (SD 0.59); range of pH 5.1 - 7.4.

4.6.2 Total duodenal bulb acid exposure

The total acid exposure of the duodenal bulb during the 24 hours of the study was determined using the "Frequency Distribution" analysis. The cumulative percentage of the total number of recordings made by the duodenal electrode, to levels of pH less than 5, 4, 3, 2, and 1 were analysed, and the results of daytime and nocturnal exposure were compared.

Table 4.1 shows the cumulative pH exposure in the duodenal bulb at pH levels 1.0 to 5.0. The acid exposure at each pH level was similar during the day and at night, and the incremental increase to pH 5.0 was slightly exponential. There was no significant difference encountered between the daytime and nocturnal exposure. However, there was a tendency for a more acidic milieu to be present during

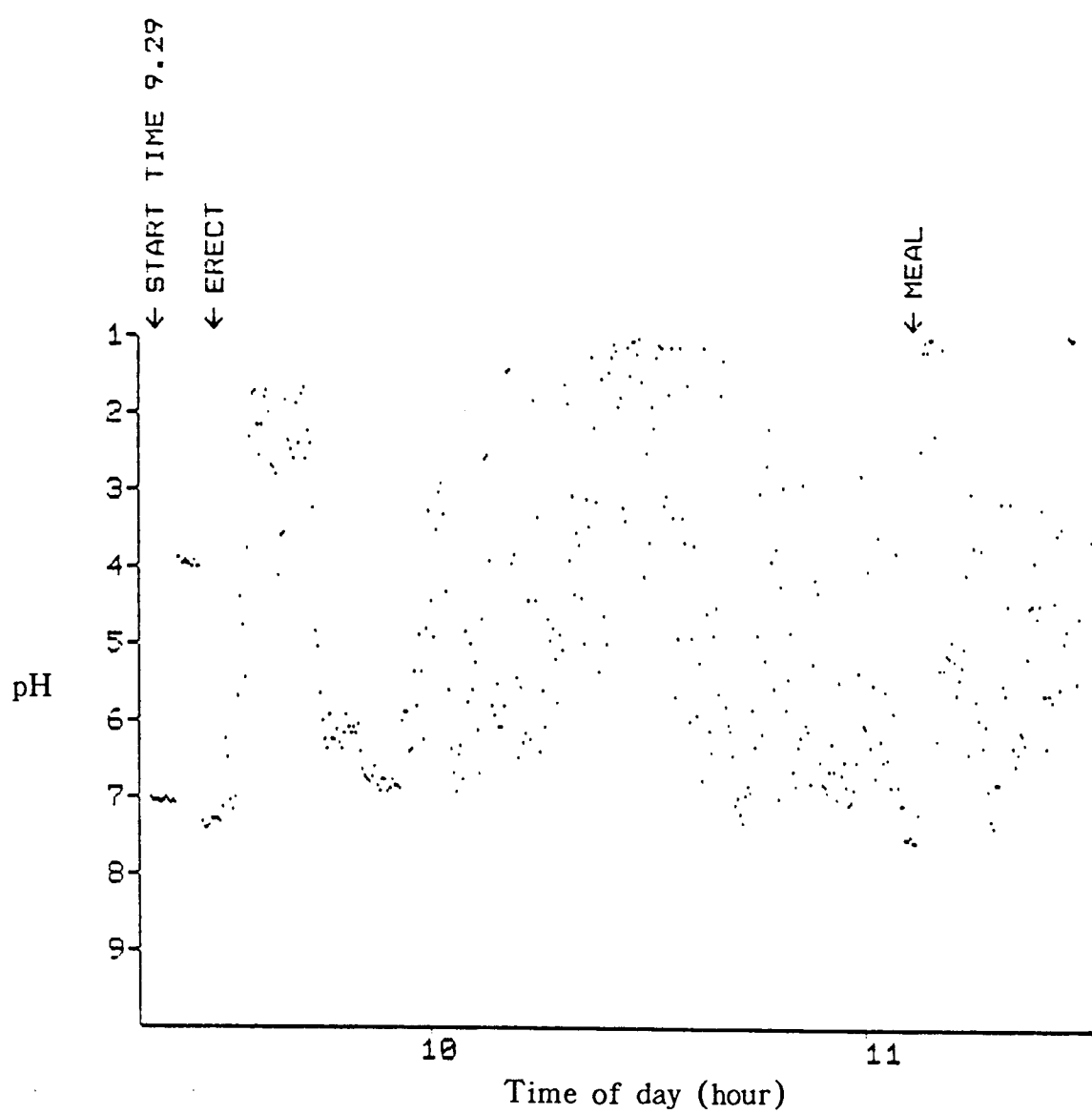


Figure 4.2 : Duodenal pH during the fasting period of a healthy subject

the day rather than at night. This data is illustrated graphically in the next chapter (Figure 5.1).

Table 4.1: Cumulative percentage pH exposure in the duodenal bulb in healthy subjects.

CUMULATIVE PERCENTAGE* OF TOTAL DUODENAL pH DATA			
pH LEVEL	DAY	NIGHT	p Value**
<1	0.4 (0.0 - 15.0)	0.5 (0.0 - 28.5)	NS
<2	8.4 (0.0 - 31.8)	4.5 (0.2 - 42.6)	NS
<3	14.6 (0.1 - 42.2)	12.8 (2.2 - 61.0)	NS
<4	23.9 (1.0 - 60.6)	25.4 (6.9 - 65.9)	NS
<5	39.6 (7.1 - 80.9)	37.7 (12.6 - 71.4)	NS

* median, range

** Wilcoxon Matched-Pairs Signed-Ranks test

4.6.3 Acid peaks within the duodenal bulb

As previously discussed in Chapter 3, an acid peak within the duodenal bulb was defined as being two or more consecutive readings from the duodenal electrode, below a particular pH level. The acid peak ended when two consecutive readings occurred above the pre-determined pH level. By altering the pH level on the computer, detailed analyses of these acid peaks were available.

The analyses showed that the time spent in an acid environment (levels of below pH 4, 3 and 2) during these peaks was similar during the day as experienced at night. There were no statistical differences demonstrated at any level. The medians and ranges of the time spent below these pH levels are shown in Table 4.2.

Table 4.2: Acid peaks within the duodenal bulb below pH levels of 2, 3 and 4 during the day and at night in healthy subjects.

pH LEVEL	TIME (minutes) (median,range)		p Value*
	DAY	NIGHT	
<2	4.6 (0.0 - 23.8)	2.1 (0.1 - 50.1)	NS
<3	7.5 (0.0 - 37.6)	6.0 (0.9 - 51.0)	NS
<4	13.2 (0.2 - 51.3)	13.4 (3.6 - 52.0)	NS

* Wilcoxon Matched-Pairs Signed-Ranks test

A more detailed analysis of the acid peaks is shown in Table 4.3. Having set the baseline pH at 4.0 (data below this level indicating an acid environment), this analysis revealed that the pattern of acid peaks within the duodenal bulb was similar during both day and night. The number and characteristics of these peaks did not differ significantly during these periods.

Table 4.3: Acid peak analysis to below pH 4.0 in healthy subjects.

	DAY	NIGHT	p Value*
Time (min.)	13.2	13.4	NS
pH <4	(0.2 - 51.3)	(3.6 - 52.0)	
Number of peaks	3.6	3.4	NS
(per hour)	(0.1 - 7.6)	(0.9 - 5.2)	
Duration per event	2.8	2.9	NS
(min.)	(1.3 - 8.6)	(1.3 - 53.3)	
Number of peaks	2.9	3.2	NS
shorter than 5 min.	(0.1 - 6.2)	(0.4 - 4.1)	
Number of peaks	0.5	0.6	NS
longer than 5 min.	(0.0 - 2.2)	(0.0 - 1.5)	

(Data standardized to one hour)

*Wilcoxon Matched-Pairs Signed-Ranks test

The above findings showed that there was no significant difference in the overall acid exposure of the duodenal bulb during both day and night. The frequency of acid exposure and the analysis of acid peaks were similar for the daytime and nocturnal recordings. This posed the question that: Was there, in fact, a difference in the pattern of the acid exposure within the bulb? In order to address this problem, the computer software was modified to analyse the number of acid peaks that were greater than, and less than, specified durations of time. The durations of the acid peaks selected for this part of the study were 1, 2, 3, 4 and 5 minutes. As performed earlier, the daytime and nocturnal data were analysed separately.

The modified version of the software programme was only available towards the end of this investigation. Thus, due to technical difficulties with hard disc as outlined in Section 3.5.1, the records of only nine of the healthy subjects were able to be analysed.

The numbers of short-lived acid peaks were first considered. These peaks were those of durations of less than 1, 2, 3, 4 and 5 minutes. The number of acid peaks of less than three minutes' duration that occurred during the day was the same as that which occurred at night. Peaks of less than two and less than one minutes' duration were slightly more frequent during daytime compared to nocturnal recordings. The opposite occurred with acid peaks of less than four and less than five minutes' duration, in that the nocturnal peaks were slightly more frequent than those during the day. There was, however, no significant difference between the daytime and nocturnal data at any of the five measured levels (Wilcoxon Matched-Pairs Signed-Ranks test). Table 4.4 shows the medians and ranges of the number of acid peaks that occurred at the various durations of time. The results are also shown in Figure 4.3 and repeated in Figure 5.3 in Chapter 5.

Marked and significant differences were noted when the numbers of short-lived and long-lived acid peaks were compared (Table 4.4). During daytime recordings, the short-lived peaks were more frequently encountered than long-term peaks. Using statistical comparisons with Wilcoxon Matched-Pairs Signed-Ranks test, these differences were significant ($p < 0.01$ and $p < 0.02$). However, this was only the case for peaks shorter than / longer than 2, 3, 4 and 5 minutes. The situation was reversed for the peak duration of one minute. There were significantly ($p < 0.01$) greater numbers of acid peaks lasting more than one minute, than those lasting less than one minute. Analysis of

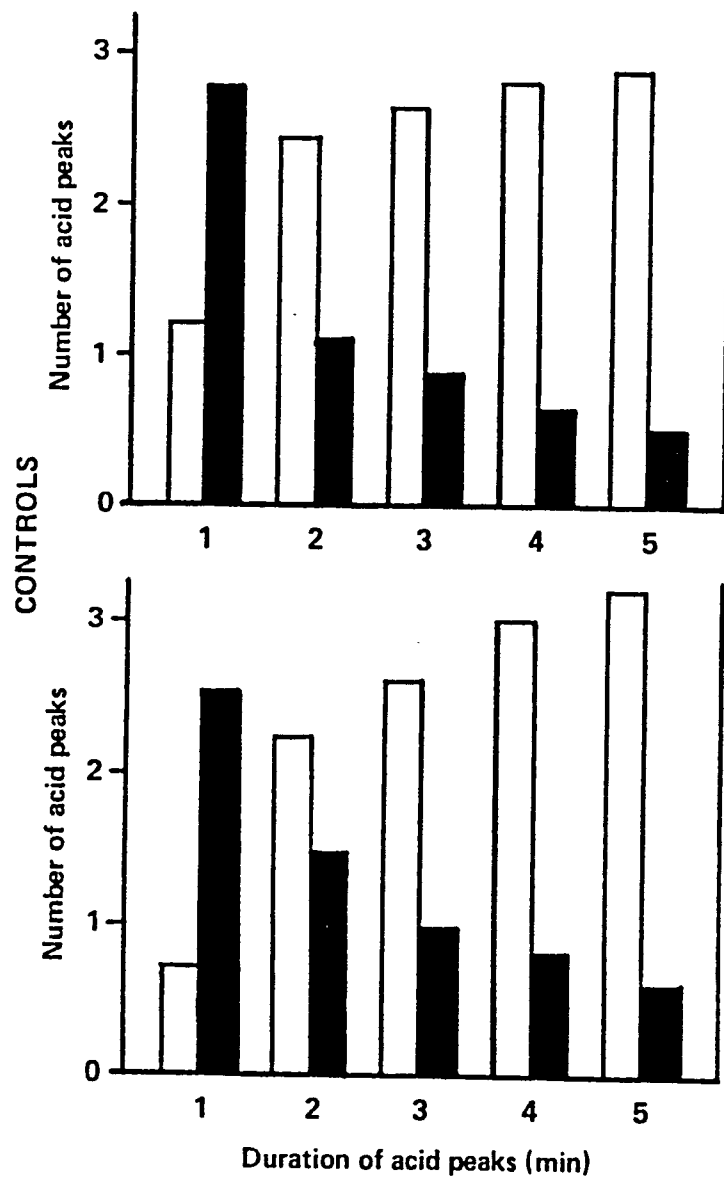
the nocturnal recordings produced very similar results. There were significantly ($p < 0.01$) more acid peaks of short duration over the range of 3, 4 and 5 minutes (significance was not reached for the duration of 2 minutes), and significantly ($p < 0.01$) greater numbers of peaks longer than one minute. These results are displayed graphically in Figure 4.3, and repeated in Chapter 5 (Figure 5.4).

Table 4.4: Analysis of acid peaks of varying duration within the duodenal bulb in nine healthy subjects.

Duration (min.)	NUMBER OF ACID PEAKS**				p* short/long	
	shorter than		longer than		day	night
	Day	Night	Day	Night		
1	1.2	0.7	2.8	2.5	<0.01	<0.01
	(0.3-1.7)	(0.2-1.8)	(1.3-6.1)	(2.1-4.4)		
2	2.4	2.2	1.1	1.5	<0.02	
	(0.7-4.6)	(0.7-3.4)	(0.3-4.1)	(0.6-3.1)		
3	2.6	2.6	0.9	1.0	<0.01	<0.01
	(0.8-3.7)	(0.8-5.2)	(0.1-3.1)	(0.5-2.0)		
4	2.8	3.0	0.6	0.8	<0.01	<0.01
	(0.9-5.4)	(0.8-4.1)	(0.1-2.5)	(0.3-1.8)		
5	2.9	3.2	0.5	0.6	<0.01	<0.01
	(0.1-6.2)	(0.4-4.1)	(0.0-2.2)	(0.0-1.5)		

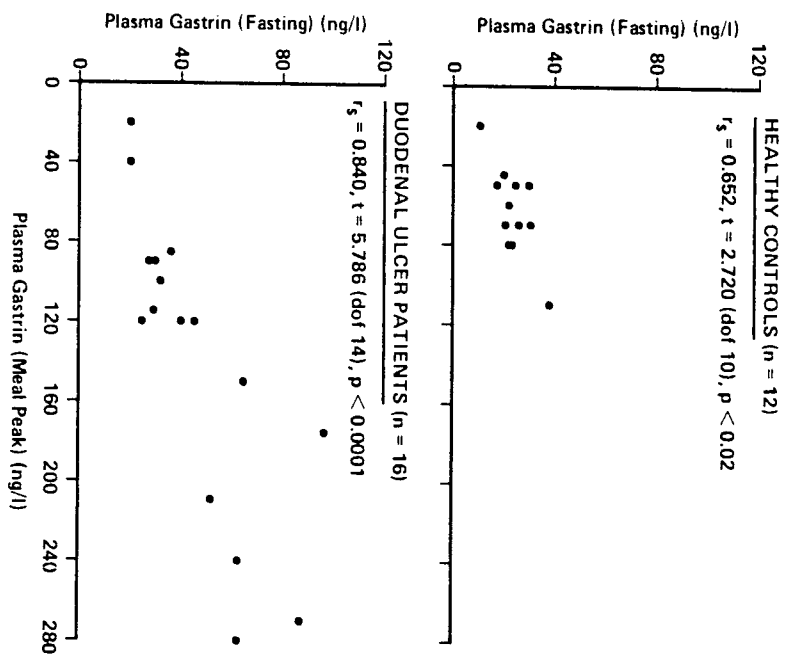
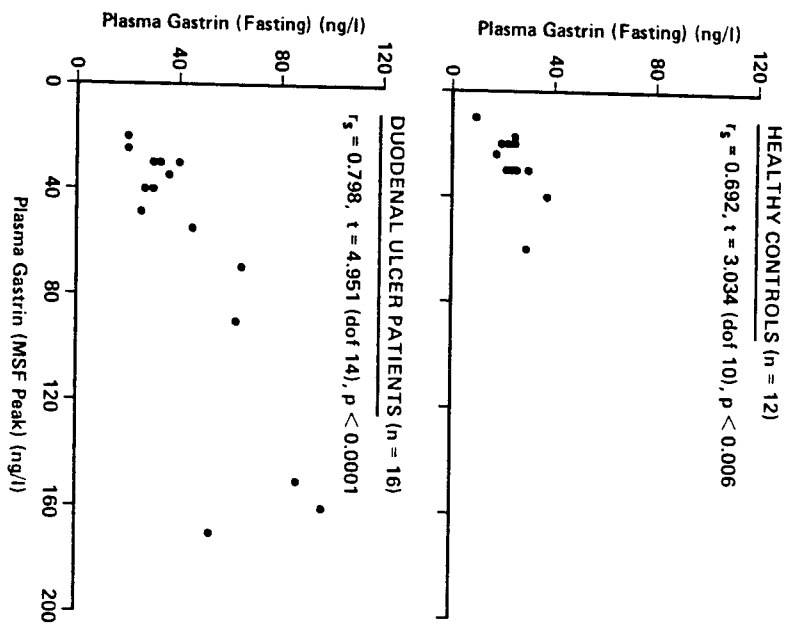
**Medians, ranges; data standardized to one hour

*Wilcoxon Matched-Pairs Signed-Ranks test



Comparison of
Short-lived to
Long-lived Acid
Peaks in Duodenal
Bulb of Healthy
Subjects

Figure 4.3



4.7 NORMAL GASTRIC pH PROFILE

4.7.1 Fasting Gastric pH

In contrast to the pH fluctuations seen in the duodenum, the fasting gastric pH was relatively constant Figure 4.4. The same pattern was exhibited in all the subjects. As for the fasting duodenal pH, the fasting gastric pH was determined by the mode of the pH data recorded during the first hour of the study (i.e. the fasting state). The mean gastric pH during this period was pH 1.91 (SD 0.33); range from 1.3 to 2.4.

4.7.2 Intragastric acidity

Analysis of the data from the gastric pH electrode was performed in a similar fashion as for the duodenal pH data. The pH profile of the gastric antrum was somewhat more acidic at night than exhibited during the day. The buffering effects of meals and drinks would explain these differences, which were statistically significant at pH 3 and 4. Table 4.5 shows the frequency distribution of the gastric pH as the cumulative percentage of the total gastric pH data.

An alternative method of examining the pH profile within the gastric antrum, is shown by the analysis of the duration of pH rises in the antrum (Table 4.6). A pH rise was defined as two or more consecutive data points above a pre-determined pH level, and was completed by the first two consecutive data points to occur below the same pH level. The time spent during these pH rises demonstrated a pH profile similar to that shown by the cumulative pH percentage analysis. The daytime exposure was longer than that experienced at

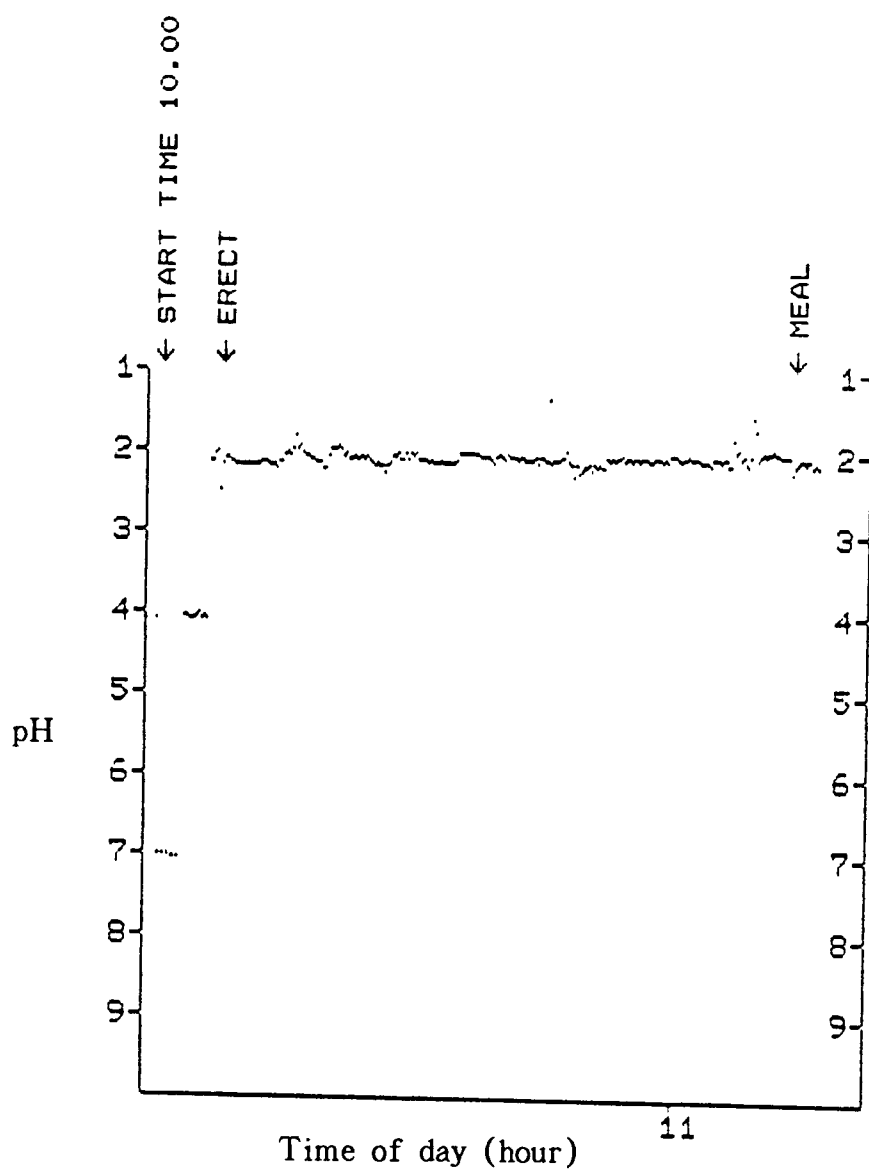


Figure 4.4 : Antral pH during the fasting period

Table 4.5: Cumulative pH percentage of gastric pH data in healthy subjects.

CUMULATIVE PERCENTAGE* OF TOTAL GASTRIC pH DATA			
pH LEVEL	DAY	NIGHT	p Value**
>1	100.0 (48.7 - 100.0)	100.0 (71.2 - 100.0)	NS
>2	61.9 (19.4 - 99.1)	47.6 (5.0 - 100.0)	NS
>3	26.4 (11.4 - 56.5)	14.4 (0.4 - 28.8)	<0.002
>4	11.4 (3.5 - 29.3)	2.8 (0.3 - 16.5)	<0.001
>5	2.5 (0.3 - 16.5)	1.6 (0.0 - 10.1)	NS
>6	0.3 (0.0 - 9.3)	0.0	NS

*median, range

**Wilcoxon Matched-Pairs Signed-Ranks test

night, and the differences reached significance ($p < 0.01$) at the levels of $\text{pH} > 3$ and $\text{pH} > 4$.

Table 4.6: Duration of rises in intragastric pH to above pH levels of 1, 2, 3, 4 and 5 in healthy subjects.

TIME DURING pH RISES*			
pH LEVEL	DAY	NIGHT	p Value**
>1	59.2 (36.4 - 59.7)	59.9 (28.2 - 59.9)	NS
>2	39.4 (10.2 - 58.2)	24.6 (2.4 - 59.9)	NS
>3	15.1 (6.3 - 30.7)	6.8 (0.1 - 14.0)	<0.01
>4	6.6 (1.9 - 21.8)	1.6 (0.0 - 9.7)	<0.01
>5	1.7 (0.0 - 16.9)	1.0 (0.0 - 5.7)	NS

*Median, range; Data standardized to one hour

**Wilcoxon Matched-Pairs Signed-Ranks test

The intragastric pH level was generally stable at the baseline pH and rose on ingestion of food or drink. Figure 4.5 illustrates the rise in gastric pH following a meal. The level to which the pH rose was dependent upon the constituents of the meal and its volume. During nocturnal recordings, the pH remained steady until morning. However, six subjects demonstrated spontaneous rises in the intragastric pH (Figure 4.6). Five subjects exhibited a single such episode, whilst the sixth subject showed three episodes of pH rise. In each case, there

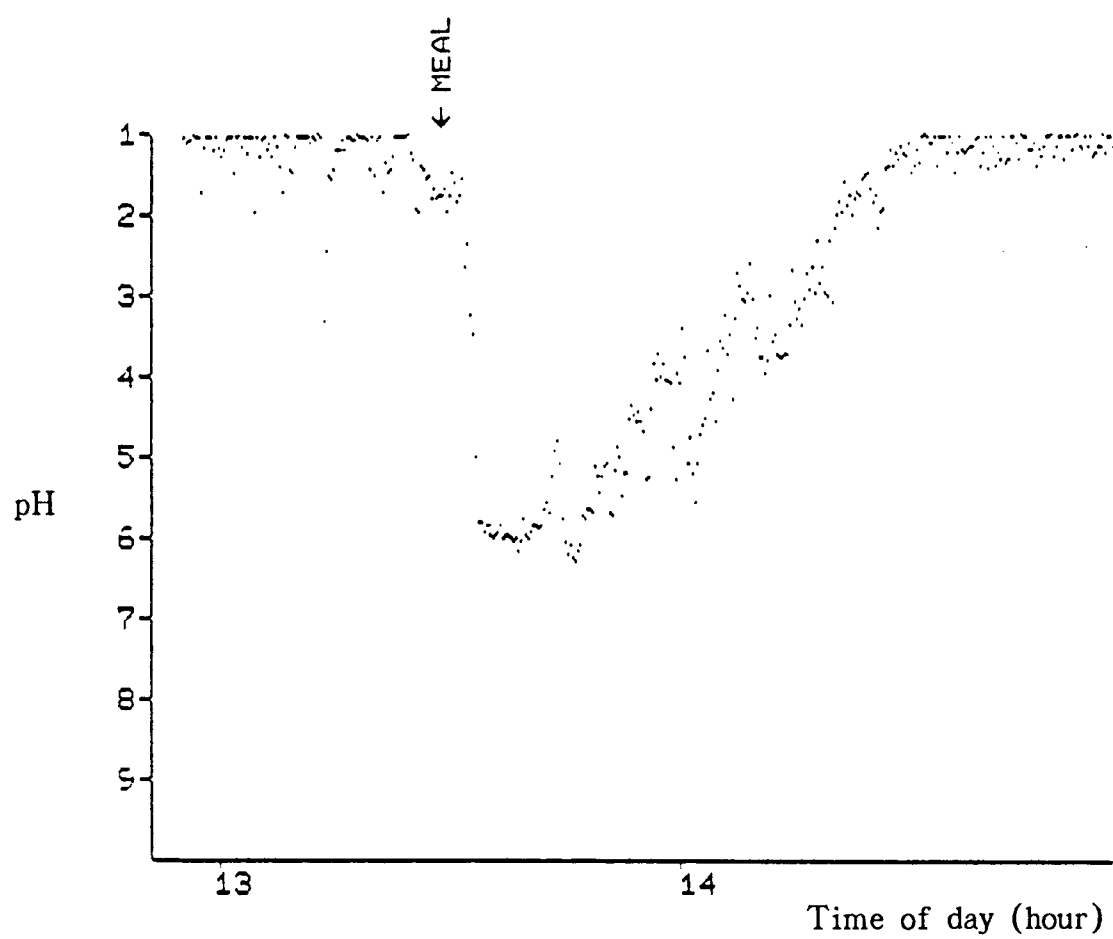


Figure 4.5 : Effect of ingestion of a meal on antral pH



Figure 4.6 : Recording of antral pH at night showing spontaneous rises in pH of varying duration

was no apparent precipitating cause for these pH rises, and prolapse into the duodenal bulb was ruled out as the concurrent duodenal pH tracing showed no sudden change in pattern. These pH peaks occurred between 1.00 am and 6.30 am, and lasted between seven minutes and two hours, with a median of 20 minutes. The extent of each rise ranged from pH 4.5 to pH 7.5 (median pH 6.7).

4.8 THE EFFECT OF MODIFIED SHAM FEEDING AND AN INGESTED SOLID MEAL*

The ingestion of the meal produced an initial rise in the gastric pH which reached a peak level of mean pH 5.3 (SD 0.7). This was followed by a gradual decrease in pH (rise in intra-gastric acidity) to return to the pre-meal pH level. The duration was variable but had a median value of 98.0 minutes (range 59.5 - 130.5 minutes). The effects, on the duodenal bulb pH, of cephalic stimulation by modified sham feeding and the ingestion of a solid meal were analysed together, and the remaining data of the 24 hours was excluded.

Following the two feeds, the exposure to acid in the duodenal bulb was determined by the cumulative pH percentage analysis. This yielded the cumulative percentage of the frequency distribution of the total pH data during this period. The overall acid exposure was similar to that experienced during the full duration of the study (viz. Table 4.1), although the ranges of pH values were slightly wider. These results are shown in Table 4.7.

* Due to computer programme difficulties, the results of the two meals were analysed together.

Table 4.7: Acid exposure in the duodenal bulb following cephalic stimulation and a meal in healthy subjects.

CUMULATIVE PERCENTAGE	
<u>pH LEVEL</u>	<u>OF TOTAL pH DATA*</u>
<2	5.3 (0.0 - 36.7)
<3	17.2 (0.0 - 53.1)
<4	28.8 (0.9 - 78.8)

*Median, range

The pattern of acid peaks that occurred during this period was almost identical to that experienced during the full 24 hour study (viz. Table 4.3). The time spent in an acidic milieu (pH <4.0), the number of acid peaks per hour and their average duration were remarkably similar to that seen in the overall pattern. Only the duration of peaks showed a wider range. The results of this analysis are shown in Table 4.8.

Table 4.8: Analysis of acid peaks within the duodenal bulb following cephalic stimulation and a meal in healthy subjects.

<u>PARAMETER</u>	<u>Median (range)</u>
Time* pH <4	15.0 (0.1 - 46.7)
No. acid peaks/hour	3.7 (0.1 - 7.8)
Duration* per peak	2.9 (0.7 - 18.9)
No. peaks <5 min.	3.0 (0.2 - 6.4)
No. peaks >5 min.	0.5 (0.0 - 1.5)

*minutes; Data standardized to one hour

4.9 DISCUSSION

This study shows that, with the improved technology and methods, long-term ambulatory dual gastro-duodenal pH monitoring is now possible. For the first time, an accurate profile, in normal healthy subjects, of the twenty four hour acid exposure within the duodenal bulb has been recorded, and detailed assessment of the differences between daytime and nocturnal exposure can be made. These results enable new light to be shed on the physiology of the duodenum. In addition, the concurrent gastric pH profile allows for better understanding of the pH fluctuations in the duodenum.

The problems associated with pH measurements within the duodenal bulb have been referred to in Chapter 2 and previously discussed by a number of authors (Rovelstad and Maher 1962, Andersson and Grossman 1965, Rune 1981). The most important of these factors is placing and maintaining the electrode within the duodenal bulb at a known and reproducible distance from the pylorus. This is vital due to the steep pH gradient between the apex and the base of the bulb (Rhodes and Prestwich 1966, Rune 1981). Measurement of the change in transmural electrical potential difference across the pylorus was proposed for identifying the gastro-duodenal junction (Andersson and Grossman 1965). Using this method, the position of the duodenal electrode was established. However, maintaining its position was the drawback of this system and frequent repositioning of the electrode was not practical over the longer term. An alternative proposal was the use of four electrodes strapped together at 1.5 cm. intervals (Rune 1981, Hannibal and Rune 1983). A four-channel analogue pH recorder displayed the four pH tracings, and the pattern of tracings enabled the

positions of each electrode to be determined. Although accurate, the system was too bulky to be portable and not suitable for long-term recordings. The method employed in this present investigation was relatively simple and ensured that the electrode was placed at a reproducible distance from the pylorus. The apex of the duodenal bulb was selected as the site for pH determination as it was the more readily apparent site on the fluoroscopic screen. Accurate placement was achieved by using the combination of voltage read-outs from the receiving unit and fluoroscopic screening, as earlier described. It has been shown earlier (McCloy, Vickery and Baron 1980) that the caudal peristaltic pull on the weighted bag, which acted like a sheet-anchor, maintained the electrode position most adequately in the short-term (five hours) and this was confirmed during the 24 hours of this study. In only five of the forty eight studies was shift of the electrode position noted.

The mean fasting pH in the duodenal bulb of this cohort of healthy subjects was pH 6.73, and the individual values ranged from pH 5.1 to pH 7.4. These results are in agreement with those of other studies (Berk, Rehfuess and Thomas 1942, Schaffalitzky de Muckadell et al 1979, McCloy, Greenberg and Baron 1984) which reported a range of mean duodenal bulb pH of 5.0 - 7.0. However, other studies involving normal subjects have reported lower mean fasting pH values in the bulb (Archambault, Rovelstad and Carlson 1967, Rune 1968, Hannibal and Rune 1983, Bendtsen et al 1986, Ovesen et al 1986). These latter reports found the mean bulb pH to vary between pH 2.4 and pH 4.5. The discrepancy between the two sets of values arises from the differing positions of the recording electrodes. Rune's unit in Copenhagen has always measured and reported the pH within 1.5 cm. of the pylorus and lower pH values have always been found in this

area. Nevertheless, the fourth (most distal) electrode of their monitoring system was situated in the distal part (apex) of the duodenal bulb. This electrode recorded a fasting pH of approximately 6.5 (Hannibal and Rune 1983).

The pattern of fasting pH values in the present study was varied, and showed multiple rapid fluctuations towards higher acidity. The median values in all the subjects remained in the pH range of 5.0 - 7.0. By increasing the sampling rate, the short duration of these fluctuations was accentuated, as can be seen by comparing Figure 3.9 with Figure 4.2. The presence of these fluctuations were noted many years ago and caused consternation as to the validity of the measured pH. Because of this, the use of shields around the electrodes was proposed. However, the pH fluctuations are considered to be a reflection of the intense acid neutralization process that occurs in the bulb following the intermittent emptying of gastric contents, especially after a meal.

The fasting gastric pH of 1.91 (range 1.3 - 2.4), demonstrated in this study, compared favourably with those levels reported previously: a range from pH 1.0 to pH 3.9 (Berk, Reh fuss and Thomas 1942, Rovelstad, Owen and Magath 1952, Rhodes and Prestwich 1966, Fimmel et al 1985, Oveson et al 1986). In contrast to the duodenal pH, no fluctuations were observed in gastric pH during the fasting period, and this confirms previous reports. The electrode in this investigation was placed in the antrum along the lesser curve. This point is important for comparisons with other studies, as it has been established that the gastric fundus has a lower pH than the antrum (Tomenius and Williams 1960, Marcussen and Rygvold 1961). However, recently it

was found that the fasting antral pH was similar to that of the fundal pH (Fimmel et al 1985).

There have been no reports in the literature of the analysis of 24 hour pH monitoring of the gastro-duodenal contents. Rhodes, Apsimon and Lawrie (1966) investigated a small group of six normal subjects for between four and fifteen hours. As the results are all analysed together, it is not possible to construct a pH profile. More recently, a new technique (McCloy, Vickery and Baron 1980) was described for dual pH monitoring, but results from a group of healthy subjects was not reported.

The profile of the duodenal bulb exposure to acid during the day took on an exponential shape where almost 40% of the pH readings occurred below pH 5.0 and approximately 8% were below pH 2.0. Although wide individual variation occurred, the trend in exposure followed the same downward curve. Of interest, was the comparison of this daytime profile with that which occurred at night. The nocturnal acid exposure was found to be of similar profile as the daytime curve: almost 38% of the data occurred below pH 5.0 and around 4% at a level of below pH 2.0. The upper limit of the range of nocturnal exposure was skewed slightly by the acidic exposure experienced by one of the subjects. Nevertheless, there appeared a tendency for the daytime exposure to be somewhat more acidic than that at night. This latter tendency could be expected as the interdigestive secretory rate of gastric acid is normally low at 2 - 3 mEq/hour. In contrast, patients with duodenal ulcer were found to have significantly increased nocturnal gastric secretory rates (Winkelstein 1935, Levin, Kirsner and Palmer 1950).

The pattern of acidity within the antrum differed during the daytime recording from that at night. Although the mean pH ranged from pH 1.5 to pH 2.5, rises in antral pH during the day occurred only at times of eating or drinking, and on occasions, reached a level of pH 6.0. On the other hand, at night, only a minority of recordings rose to a level of above pH 3.0. Indeed, the exposure at night to levels above pH 3.0 and 4.0 were significantly less than the daytime exposure. Although the nocturnal gastric pH was usually constant, six subjects exhibited spontaneous rises in antral pH to between pH 4.5 and pH 7.5. These episodes lasted between seven minutes and two hours. As no apparent precipitating cause was encountered, the possibility of duodeno-gastric reflux should be considered. These pH rises have been noted by other workers (Shiratori et al 1983, Fimmel et al 1985, Kapur 1987 [personal communication]). Previous studies have encountered a decline in early morning acidity and its increase before breakfast (Sandweiss et al 1946, Fimmel et al 1985), but this was not found in the present study.

As previously mentioned, the pH pattern in the duodenal bulb is one of "background" near-neutrality interrupted by rapid and frequent peaks of acidity. The pattern of this fluctuating acidity was similar during the day as it was at night. However, longer acid exposure was demonstrated during the day. This slight increased exposure could be expected due to the daytime ingestion of meals, and hence increased gastric acid secretory rates. The acidity within the duodenal bulb depends upon three main factors: rate of inflow (gastric emptying rate, retropulsion from the second part of the duodenum); neutralization processes; and rate of outflow (duodenal propulsive motility). As the number of acid peaks and their average duration did not vary between day and night, it could be speculated that the gastric

emptying rate is similar during day and night. Interestingly, rises in duodenal acidity post-prandially have been demonstrated without concurrent rises in antral pressure (Rhodes, Goodall and Apsimon 1966). The neutralization process and duodenal propulsive motility can be examined by closer inspection of these acid peaks. For durations of two or more minutes, the number of short-lived acid peaks significantly outnumbered the number of long-lived peaks, both during the day and at night (see Table 4.4). However, with the duration set at one minute, there were significantly more long-lived peaks. This latter finding could be a result of the sampling rate being too slow. A faster rate would have detected the rapid and brief peaks already described. An example of this is shown in Figure 3.9 which illustrates the fasting duodenal pH sampled at a rate of once every second. However, the importance of these extremely rapid acid peaks in the physiology of the duodenum remains unclear. The study by Wormsley and Mahoney (1967) showed that in healthy subjects, adequate amounts of bicarbonate were secreted in response to exogenous secretin and acid stimulation. They suggested that intraluminal buffering with bicarbonate was the main mechanism for the removal of hydrogen ions from the duodenum. This also suggests an explanation for the rapid and brief peaks of acidity. As the ratio of short- to long-lived peaks was the same during day and night, it suggests that the duodenal propulsive motility is the same during day and night.

It is well established that gastric acid secretion is stimulated by vagal activity (Mayer et al 1974, Stadil 1974, Feldman and Walsh 1980, Brunner et al 1984), the mechanism of which is thought to be primary direct stimulation of the parietal cells (Mayer et al 1974, Richardson et al 1977, Konturek et al 1978). Vagal Stimulation alone is the result of cephalic stimulation which encompasses thought, sight, smell and

taste of food. It has been found that thought is the most powerful of these stimulating factors (Feldman and Richardson 1986). Two methods may be employed to stimulate vagal activity, either pharmacologically with insulin-induced hypoglycaemia, or physiologically with either adequate sham feeding or modified sham feeding. The former feed allows the normal consumption of a meal that is not allowed to reach the stomach (food is diverted away through a gastrostomy). The latter feed uses the "chew-and -spit" technique. These two feeding methods have been compared (Stenquist, Knutson and Olbe 1978) and both produced similar acid responses. Modified sham feeding was used in this investigation for a feed duration of thirty minutes, as this has been shown to be the optimum time (Konturek et al 1981).

The combination of cephalic stimulation by modified sham feeding and the ingestion of a solid meal produced a duodenal exposure to acid that was slightly greater than the full daytime exposure. This would be expected with the increased gastric acid secretion. These figures compare with some previous reports (Berk, Rehfuess and Thomas 1942, Hannibal and Rune 1983, Ovesen et al 1986), but not others who showed less acidic post-prandial results (Rhodes and Prestwich 1966, McCloy, Greenberg and Baron 1984). The pattern of acid peaks within the bulb did not change during this post-prandial period. Of particular note, is the similar number of recorded acid peaks. Hannibal and Rune (1983) have also reported the numbers of peaks pre- and post-meal to be similar, except for during the immediate post-prandial period.

CHAPTER 5

24 HOUR AMBULATORY DUAL GASTRO-DUODENAL pH PROFILES OF PATIENTS WITH ACUTE DUODENAL ULCER

5.1 HYPOTHESIS I

The formation of duodenal ulcer is a result of an imbalance of the normal interaction between gastric juice (acid and pepsin), duodenal secretions (bicarbonate and mucus) and the duodenal mucosa. In duodenal ulcer disease, abnormal gastric hypersecretion has been well-documented (Winkelstein 1935, Levin, Kirsner and Palmer 1950, Baron 1973, Fordtran and Walsh 1973, Wormsley 1974), but there is much overlap with normal subjects (Wormsley and Grossman 1965). Excessive amounts of acid have been found in the duodenal bulb of patients with duodenal ulcer (Rhodes and Prestwich 1966, Archambault, Rovelstad and Carlson 1967), but more recent studies have not shown this (McCloy, Greenberg and Baron 1984, Bendtsen et al 1986). As these studies have all been performed in the short-term, the hypothesis is extended to encompass the full 24 hour period that: duodenal ulcer patients either are exposed to an excessive amount of gastric acid or the exposure to a "normal" amount of acid is prolonged.

5.2 STUDY AIM

In order to test the above hypothesis, the investigation described in Chapter 4 was repeated with patients with duodenal ulcer. The study was set out to achieve two main aims:

1. To record, for the first time, the continuous, ambulatory, twenty four hour, simultaneous profile of gastric and duodenal bulb pH in patients with active duodenal ulcer, off-treatment. The study incorporated periods of fasting, cephalic stimulation and ingestion of a solid meal and the respective pH responses were recorded.

2. To compare these profiles of antral and duodenal bulb pH in the duodenal ulcer patients with the cohort of healthy subjects described in Chapter 4.

5.3 STUDY GROUP

Duodenal ulcer patients (n=27). There were eighteen men and nine women. The mean age was 45.9 years, and the range was from 23 to 75 years. All patients were diagnosed endoscopically as having active duodenal ulcers, and were randomly selected on attendance at the Endoscopy Clinic. (In most cases, the endoscopy was performed by the author). Dual gastro-duodenal pH monitoring was performed within three weeks of the upper gastro-intestinal endoscopy examination. Any treatment with antacids or gastric anti-secretory drugs was stopped at least 48 hours prior to the study, and not taken during the study period. Those patients excluded from the investigation were: a) patients who had a previous history of any form of ulcer surgery being performed; b) patients with a concomitant gastric ulcer; c) patients with other gastro-intestinal pathology; d) patients under the age of 18 years.

An additional patient (A.R.) was studied. He was 23 years of age and had, two years earlier, undergone a highly selective vagotomy for duodenal ulcer disease. He had presented to the Clinic with a recurrence of his ulcer symptoms, and an active duodenal ulcer on the anterior wall of the duodenal bulb was seen at endoscopic examination. His results, for comparative purposes, are presented separately.

5.4 STUDY DESIGN - STUDY II

The study was set out as for the investigation with the healthy subjects described in Chapter 4, Section 4.3 as shown in Figure 5.1. The dual pH monitoring system was first calibrated, then positioned in the patient's antrum and duodenal bulb in similar fashion. The same standardized meal (Illustration 4.1) was served for the modified sham feed, and great care was taken in explaining the requirements of this part of the study. The patients were instructed to perform the "chew-and -spit" technique for a duration of thirty minutes.

Figure 5.1: Study Design - Study II

start MSF		MEAL				home		end
0	1	2	3	4	5	6	7...	...24

(MSF: modified sham feed) Time (hours)

Two and a half hours after the MSF, the same standardized solid meal (Illustration 4.2) was served. After the post-prandial recording period, the patients were allowed home with the pH monitoring system in situ. The same instructions were given regarding the use of the "event" button. At the end of the twenty four hour study period, the recording was stopped, re-calibration performed and the data transferred to the computer as earlier described.

5.5 ANALYSIS AND STATISTICS

Computer analysis of the pH data was performed as described in Section 3.4.3. The statistical methods (Section 3.5) used for within group and between group analyses were the Wilcoxon Matched-Pairs Signed-Ranks test and Mann-Whitney U test.

5.6 ETHICAL APPROVAL

The protocol for this part of the investigation was approved by the Ethics Committee of the University of Dundee, and the Tayside Health Board.

5.7 DUODENAL BULB pH PROFILE IN DUODENAL ULCER PATIENTS

5.7.1 Fasting duodenal bulb pH

Of the twenty seven patients with duodenal ulcer (DU), twenty four had complete duodenal pH tracings, as the distal electrode prolapsed back into the antrum for the duration of the study in the remaining three patients. As for the healthy subjects, the fasting pH was determined by the mode of the pH data from the duodenal electrode whilst recording in the fasting state. The DU patients demonstrated a mean fasting duodenal bulb pH of 6.20 (SD 1.18). This was similar to that shown by the normal subjects. The range of pH values amongst the patients, however, was wider; this being 2.0-7.5 (Table 5.1). Using the Mann-Whitney U test for non-parametric data, there was no statistical difference between the values of the two groups.

Table 5.1: Fasting duodenal bulb pH of healthy subjects and patients with active duodenal ulcer.

CONTROLS (n=19)		DU PATIENTS (n=24)		
VOL	pH	PAT	pH	
AM	6.9	MM	6.6	p = NS
CE	7.4	GD	5.1	(Mann-Whitney
IW	6.1	DD	6.9	U test,
FA	7.1	AW	6.8	Z = 1.6875)
MD	7.2	TH	6.2	
SM	6.9	RM	6.6	
LR	7.4	RL	4.7	
WF	5.9	EB	6.1	
MZ	7.0	JF	2.0	
DB	7.2	DM	6.8	
RH	6.1	RC	6.5	
CB	6.5	JM	5.8	
GM	7.3	JS	6.3	
RL	5.1	JT	7.0	
SE	6.5	CW	6.8	
MM	6.7	DL	7.5	
NL	6.8	JT	7.1	
TM	6.7	JR	6.8	
NW	7.1	LA	5.9	
		NE	7.1	
		MB	7.0	
		MM	7.2	
		DM	5.3	
		RS	4.8	

The fasting duodenal bulb pH of Patient A.R., who had previously had a highly selective vagotomy performed and had presented with an ulcer recurrence, was pH 4.4.

5.7.2 Total duodenal bulb acid exposure

The frequency of distribution of the cumulative percentage of total pH recordings from the duodenal electrode was calculated with the software analysis as for the healthy subjects. The data to the pH levels of less than 1, 2, 3, 4 and 5 were analysed.

The acid exposure at each pH level was similar during the day and at night. These results, together with those of the control group for comparison, are displayed graphically in Figure 5.2, while the tabular form is presented in the Appendix (Table A.1). There was a tendency for the daytime exposure to be slightly greater than at night, at pH levels 4 and 5, and this was reversed at pH levels of 3 and 2, where nocturnal exposure was somewhat more acidic. However, as for the healthy subjects, there was no statistical difference in the exposure at any pH level. Comparison of this data with that of the control group, showed no significant differences at any of the pH levels (Mann-Whitney U test).

Patient A.R. (Past HSV and recurrent ulcer) experienced a far more acidic environment in the duodenal bulb during the day compared to both the other DU patients and healthy subjects. Fluctuations in the pH level were extremely rapid and frequent. At night, the fluctuations continued, although to a lesser degree, and the overall exposure to acid was less than that exhibited by the other DU patients. These results are shown below:

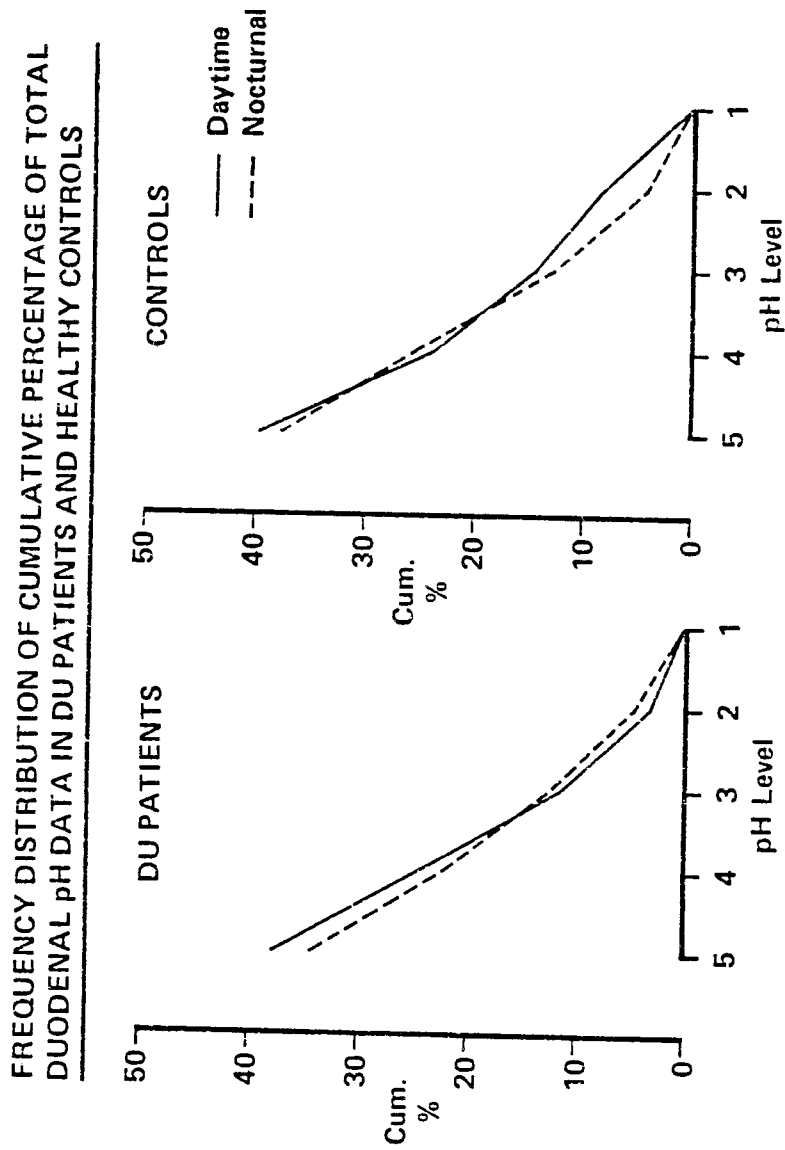


Figure 5.2 : Frequency distribution of cumulative percentage of total duodenal pH data in DU patients and healthy controls

Patient A.R.: Cumulative pH percentage of duodenal data

PERIOD	pH LEVEL:	5	4	3	2	1
DAY	%	71.9	50.9	28.4	18.2	0.2
NIGHT	%	34.7	19.7	7.4	0.4	0.0

5.7.3 Acid peaks within the duodenal bulb

Using the same acid peak analysis as described earlier, the time spent during the peaks of acidity within the duodenal bulb was considered for the pH levels of below 4, 3 and 2. Table 5.2 shows these results together with those of the controls. The acid exposure was similar during both day and night, and, as with the frequency distribution analysis, there was a tendency for the nocturnal data to be somewhat more acidic. However, there were no significant differences encountered at the three pH levels. A reversal of this pattern was seen amongst the controls. At all pH levels, the exposure to acid, as shown by this analysis, was similar in the DU patients to that in the healthy subjects, and the differences did not reach significant levels.

Table 5.2: Acid peak analysis: Time spent below pH levels of 4, 3 and 2 in healthy controls and duodenal ulcer patients.

		TIME* (minutes) (median, range)	
pH	PERIOD	CONTROLS	DU PATIENTS
<4	day	13.2 (0.2-51.3)	13.1 (0.4-29.7)
	night	13.4 (3.6-52.0)	12.0 (0.0-50.8)
<3	day	7.5 (0.0-37.6)	5.9 (0.1-22.6)
	night	6.0 (0.9-51.0)	6.7 (0.0-44.4)
<2	day	4.6 (0.0-23.8)	1.9 (0.0-17.32)
	night	2.1 (0.1-50.1)	2.1 (0.0-30.2)

*data standardized to one hour; At all levels, no significant differences were achieved either within or between each group.

During the peaks of acidity, Patient A.R. exhibited a greater daytime acid exposure (pH <4: 27.9 min.), than at night (11.8 min.)

Table 5.3 shows the more detailed analysis of acid peaks, and here the pH level has been pre-set to pH 4.0. The daytime pattern of acid peaks was similar to that exhibited at night, and the differences were not statistically significant. The 3.7 peaks per hour experienced by the DU patients by day were similar to the 3.6 peaks per hour of the controls. Overall, the patterns of acid peaks of the DU patients and healthy subjects were similar ($p = \text{NS}$, Mann-Whitney U test).

Table 5.3: Acid peak analysis to pH <4.0 within the duodenal bulb in DU patients and healthy subjects.

PARAMETER	PERIOD	CONTROLS	DU PATIENTS
Time pH <4	day	13.2 (0.2-51.3)	13.1 (0.4-29.7)
(minutes)	night	13.4 (3.6-52.0)	12.0 (0.0-50.8)
No. peaks	day	3.6 (0.1-7.6)	3.7 (0.3-9.2)
per hour	night	3.4 (0.9-5.2)	3.5 (0.0-8.1)
Duration per	day	2.8 (1.3-8.6)	3.2 (1.1-11.5)
peak (min.)	night	2.9 (1.3-53.3)	3.1 (0.0-46.6)
No. peaks	day	2.9 (0.1-6.2)	2.9 (0.0-8.2)
<5 minutes	night	3.2 (0.4-4.1)	2.8 (0.0-7.1)
No. peaks	day	0.5 (0.0-2.2)	0.7 (0.0-1.9)
>5 minutes	night	0.6 (0.0-1.5)	0.7 (0.0-2.4)

(data standardized to one hour; medians, ranges)

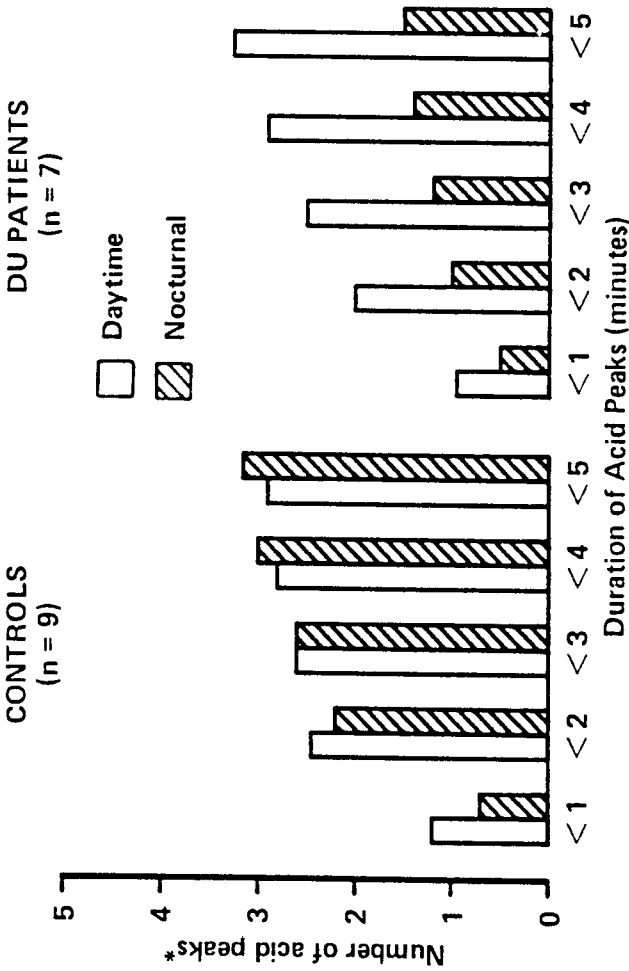
For all parameters, no significant differences were observed.

The above results showed that the patients with active duodenal ulcer experienced a similar duration and pattern of acid exposure in the duodenal bulb as did the healthy subjects. The question of whether or not there was a difference in the pattern of this exposure within the bulb was then addressed to the DU patient group. Seven records were available for additional analysis by the new modified software programme (see Section 3.4.3). The durations of the acid peaks selected here were 1, 2, 3, 4 and 5 minutes.

Figure 5.3 shows the numbers of short-lived acid peaks in the duodenal bulb for both the DU patients and controls; (short-lived peaks being less than 1, 2, 3, 4 and 5 minutes' duration). The number of short-lived peaks exhibited by the DU patients during daytime recording was similar to that of the healthy subjects, and no significant differences were observed (Mann-Whitney U test). However, during nocturnal recordings, the DU patients exhibited far fewer short-lived peaks than during the day; the differences did not reach significance, presumably because of the small numbers. Comparing these nocturnal results, there would appear to be a marked difference between the control and patient groups, but again due to the small numbers, statistical significance was not reached.

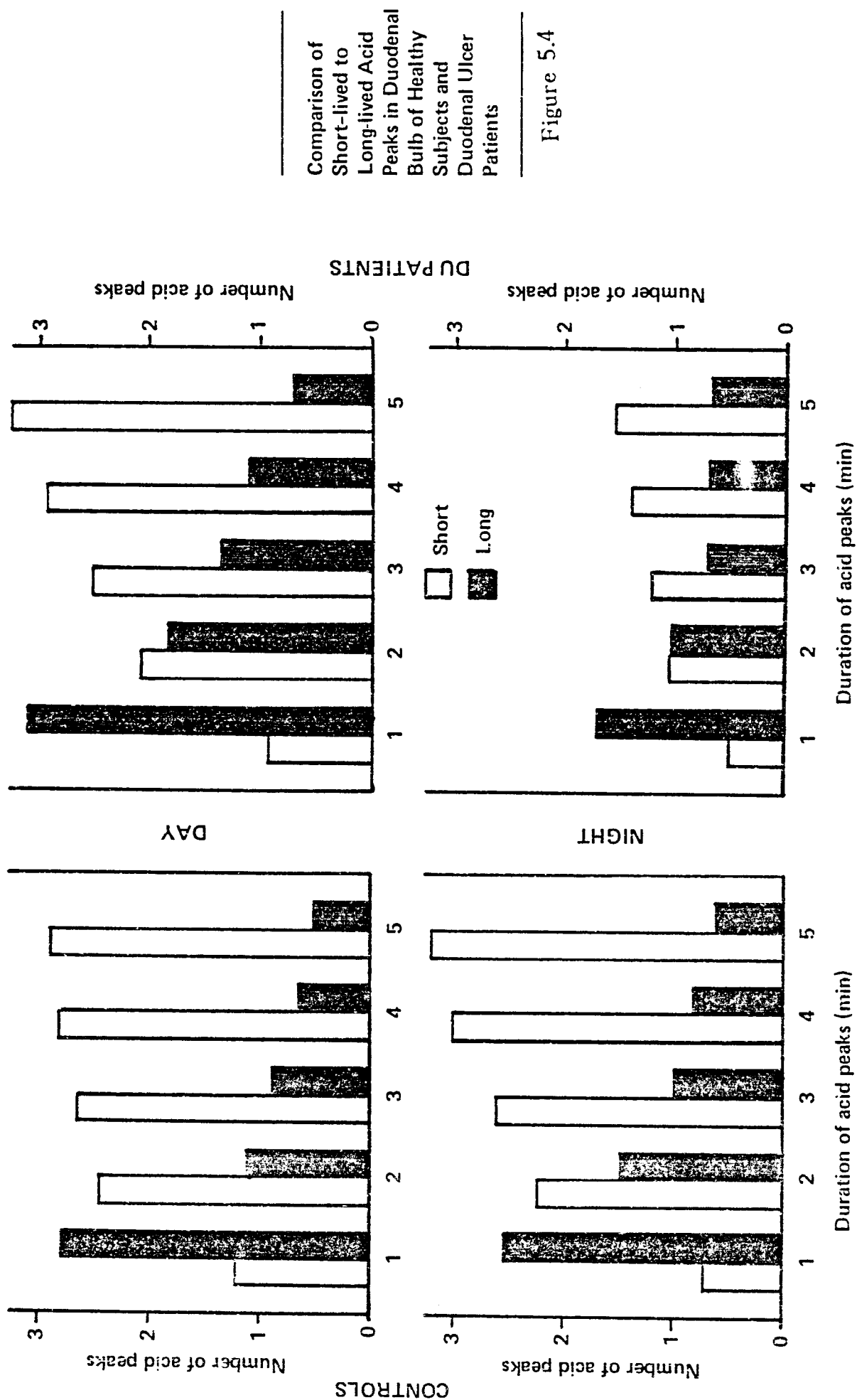
Further differences were observed by comparing the numbers of short-lived and long-lived acid peaks (i.e. shorter than and longer than the pre-determined duration). The results are shown in Figure 5.4, as well as in tabular form in Table A.2 in the Appendix. The daytime recordings revealed significantly ($p < 0.01$) more frequent short-lived acid peaks of duration less than 3, 4 and 5 minutes. This pattern was reversed for acid peaks of one minute's duration, as the long-lived peaks were present in significantly ($p < 0.01$) greater numbers. This

ANALYSIS OF NUMBER OF ACID PEAKS OF VARYING DURATION IN
HEALTHY CONTROLS AND DU PATIENTS



* Data std. to one hour.

Figure 5.3 : Analysis of number of acid peaks of varying duration (less than 1, 2, 3, 4 and 5 minutes) in healthy controls and DU patients



pattern compared favourably with that of the controls, and no significant differences were observed between the two study groups. The nocturnal recordings showed a similar pattern but somewhat scaled down. Short-lived peaks were only present in significantly ($p < 0.01$) greater numbers at the duration of five minutes. Again, long-lived peaks of greater than one minute's duration were significantly ($p < 0.01$) more numerous than short-lived acid peaks.

Patient A.R. displayed a similar pattern of short- and long-lived acid peaks, with short-lived peaks being more numerous between 2 - 5 minutes' duration, but long-lived peaks being predominant at 1 minute's duration. This daytime pattern was repeated at night.

5.8 INTRAGASTRIC ACIDITY OF DU PATIENTS

5.8.1 Fasting gastric pH

As in the group of healthy subjects, the patients with duodenal ulcer exhibited a steady gastric pH profile during the fasting period. The mean pH (mode of the data) was 1.82 (SD 0.31). The range of fasting pH levels was the same as that of the controls, being pH 1.3 - 2.4. There was no statistical difference between the fasting pH of either group (Mann-Whitney U test, $Z = 0.7701$).

The fasting gastric pH of Patient A.R. was similar to the group as a whole, at pH 1.9.

5.8.2 Intragastric acidity

The cumulative percentage of the total gastric pH data revealed an exponential decrease in the profile of the daytime exposure as the pH rose to 7.0. This profile matched that of the healthy subjects, although

the latter experienced a slightly more acidic exposure (Figure 5.5, Table A.3). The pH profile of nocturnal readings was more acidic and showed a steeper curve. Significant differences between the daytime and nocturnal recordings were observed at pH 3, 4 and 5 ($p < 0.01$, Wilcoxon Matched-Pairs Signed-Ranks test). At night, the DU patients experienced significantly ($p < 0.05$) fewer data points above pH 3.0 than the healthy subjects, and above this pH level, the number of readings was minimal.

The alternative analysis of determining the acid profile and exposure is shown graphically in Figure 5.6., where the duration of pH rises within the stomach is examined. The time spent during the pH rises was significantly longer during the day compared to at night. As shown by the cumulative pH percentage analysis, the nocturnal recordings were more acid as minimal time was spent at pH levels of above 3, 4 and 5. The time spent above pH 3 and 4 at night was significantly ($p < 0.05$) shorter among the DU patients when compared to controls. This data is also shown in Table A.4.

Patient A.R. experienced less time during the day at the higher pH levels of 3, 4 and 5 than the other DU patients. There was a sharp drop in the exposure time when considering the levels of pH >2 and pH >3 . At night, a more steady gastric tracing was obtained and the pH rarely rose to above pH 3.0.

CUMULATIVE pH PERCENTAGE OF GASTRIC pH DATA IN HEALTHY SUBJECTS
AND DUODENAL ULCER PATIENTS

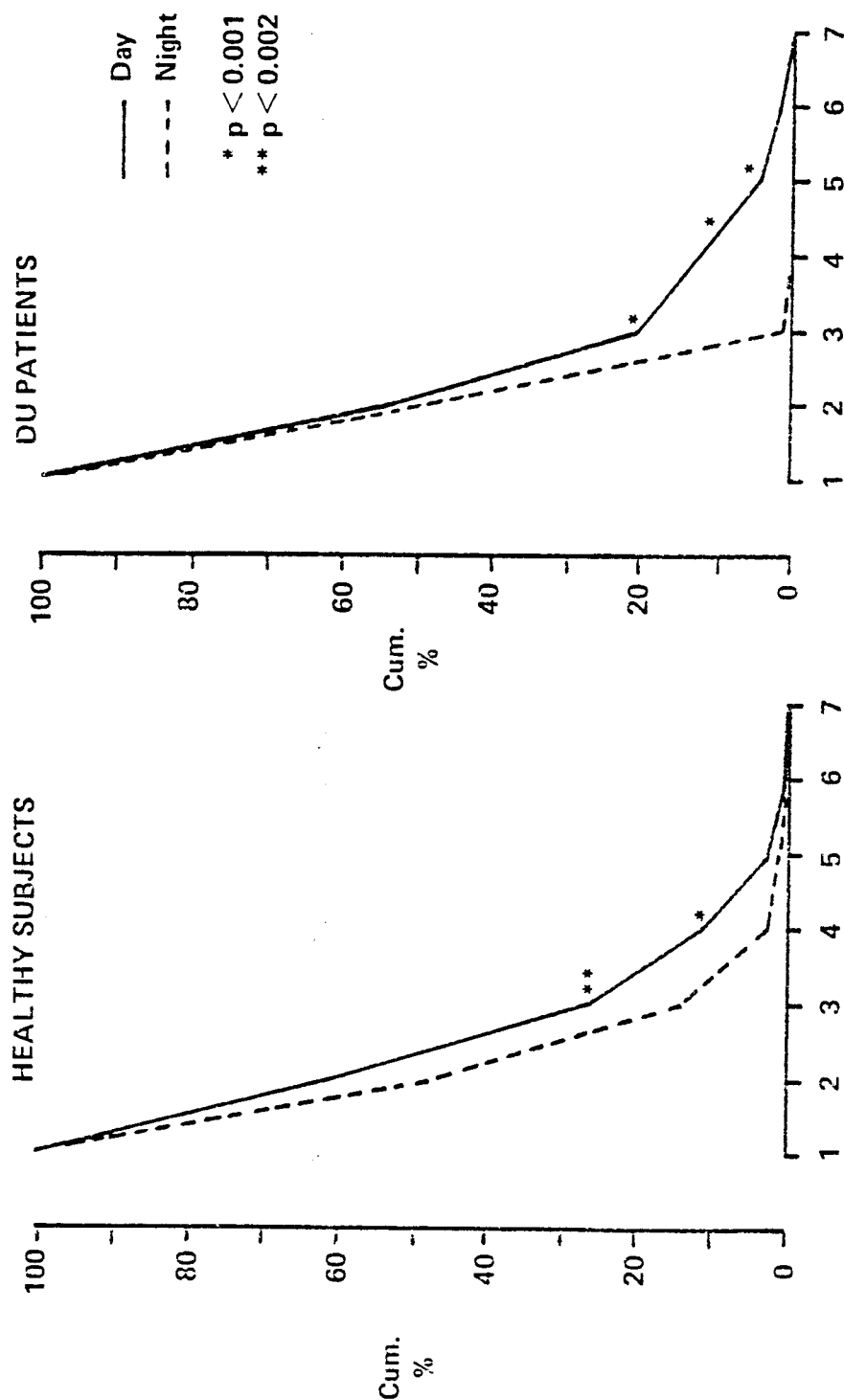
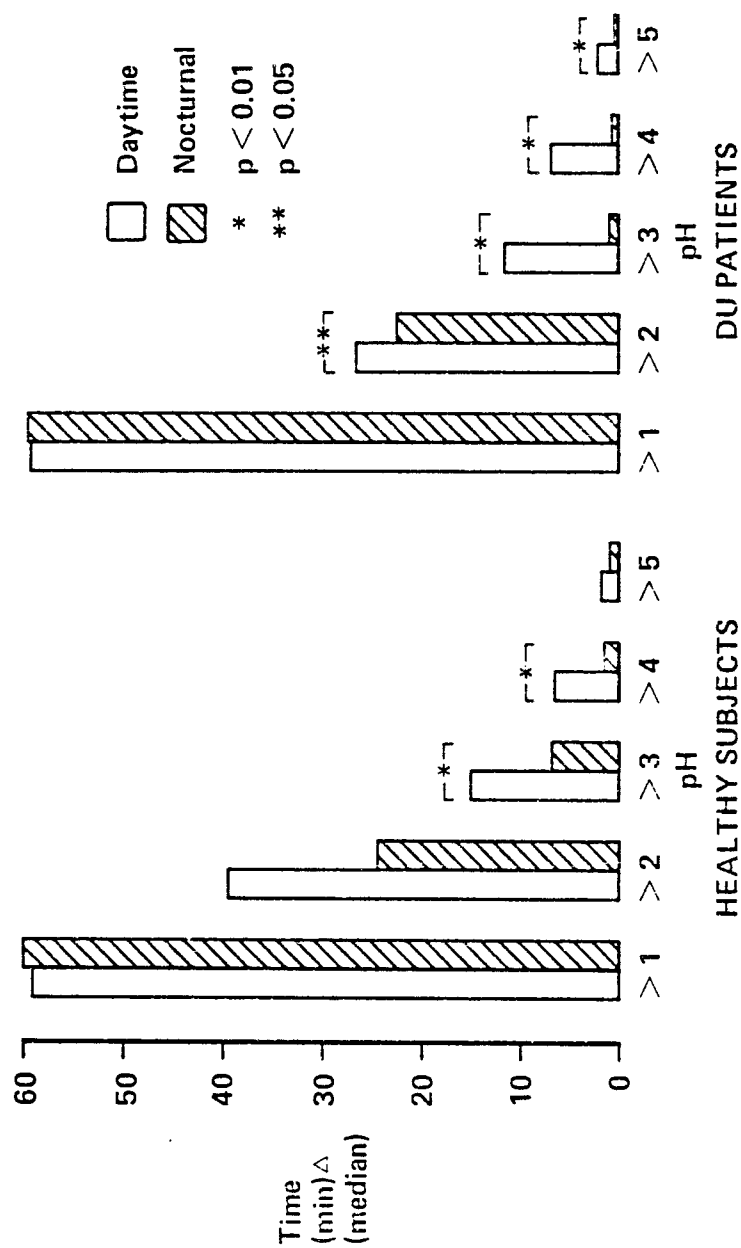


Figure 5.5

DURATION OF RISES IN INTRAGASTRIC pH OVER 24 HOURS IN HEALTHY SUBJECTS AND DUODENAL ULCER PATIENTS



Δ data standardized to one hour.

Figure 5.6

Patient A.R.: Duration of rises in intragastric pH to above pH levels of 1, 2, 3, 4 and 5. (data standardized to one hour)

<u>pH LEVEL</u>	<u>TIME (min.): DAY</u>	<u>NIGHT</u>
>1	59.5	59.9
>2	33.7	52.6
>3	9.4	0.8
>4	2.9	0.4
>5	1.2	0.2

Seven patients exhibited the spontaneous rises in nocturnal pH that were observed amongst the healthy subjects. The pH tracings at night of the remaining patients showed a steady profile. Five DU patients showed a single rise in pH and two experienced three episodes. The range of pH rise was from pH 4.2 to pH 7.5, with a median value of pH 5.5. These episodes occurred between 1.30 am. and 5.55 am. and lasted between 10 and 80 minutes. As with the control subjects, no precipitating factors were evident, and no concomitant changes were observed in the duodenal tracing.

5.9 THE EFFECT OF MODIFIED SHAM FEEDING AND AN INGESTED SOLID MEAL IN DU PATIENTS

The ingestion of the solid meal caused an initial rise in antral pH, due to the buffering effect of the food. The peak rise in gastric pH reached a mean level of pH 5.5 (SD 0.9) and thereafter, the acidity steadily increased to return to the pre-meal level. This time was variable and ranged from 38.0 to 157.1 minutes. There was no difference when compared to the controls. The acid exposure in the duodenal bulb following the modified sham feed and solid meal was similar to that experienced over the full duration of the study.

However, during the feeding period, the percentage of data below pH levels 3 and 2 were greater, indicating a more acid environment. Table 5.4 shows the acid exposure to pH levels 2, 3 and 4. (The 24 hour data is shown in Table A.1). No significant differences were observed when comparing the exposure of the DU patients with controls.

Table 5.4: Acid exposure in the duodenal bulb following cephalic stimulation and a meal in patients with duodenal ulcer.

CUMULATIVE PERCENTAGE	
<u>pH LEVEL</u>	<u>OF TOTAL pH DATA*</u>
<2	7.4 (0.1 - 29.0)
<3	13.5 (1.1 - 50.5)
<4	24.4 (2.5 - 63.5)

*Median, range

Patient A.R. experienced a considerably greater exposure to acid following the cephalic stimulation and solid meal. The cumulative pH percentages were calculated at: pH 2: 42.6%; pH 3: 51.0%; pH 4: 68.0%.

Analysis of the acid peaks that occurred during the cephalic stimulation and meal (Table 5.5) showed the pattern of peaks to be similar to that for the full 24 hour study (viz. Tables 5.2 and 5.3). The gastric stimulation caused a fewer number of acid peaks which were of somewhat shorter duration. This pattern of acidity was similar to that of the healthy subjects (Table 4.8) and no significant differences were observed.

Table 5.5: Analysis of acid peaks within the duodenal bulb following cephalic stimulation and a meal in patients with duodenal ulcer.

PARAMETER	Median (range)
Time* pH <4	13.1 (0.9 - 37.3)
No. acid peaks/hour	2.8 (0.4 - 9.1)
Duration* per peak	2.8 (1.1 - 13.2)
No. peaks <5 min.	2.2 (0.0 - 8.1)
No. peaks >5 min.	0.4 (0.0 - 1.6)

*minutes

Data standardized to one hour

A similar pattern of acid peaks was exhibited by Patient A.R. and his data occurred in the upper half of the ranges displayed by the other DU patients.

5.10 DISCUSSION

It is important to note at the outset that the patients investigated in this study suffered from acute duodenal ulcer. They were diagnosed endoscopically, and the dual pH monitoring was performed within three weeks of the endoscopic examination. This is an important consideration as it has been shown that patients with active symptomatic duodenal ulcer have different rates of acid output compared to inactive duodenal ulcer patients (Sandvik et al 1985).

(Achord 1981)

The level of fasting pH in the duodenal bulb experienced by the patients in this investigation confirmed the results of some previous

studies (Rovelstad and Maher 1952, McCloy, Greenberg and Baron 1984). Other studies have recorded more acidic fasting levels (Eyerly 1940, Atkinson and Henley 1955, Archambault, Rovelstad and Carlson 1967, Bendtsen et al 1986, Rosenkilde-Gram et al 1986). As discussed fully in the previous chapter, the discrepancy arises from the differing positions of the recording electrode within the duodenal bulb. However, a significant finding was that there was no appreciable difference in the fasting duodenal bulb pH between the DU patients and healthy subjects, except that the range of individual values was wider amongst the patients. It has been shown that the basal gastric acid output of DU patients is significantly greater than controls (Wormsley and Grossman 1965, Feldman, Richardson and Fordtran 1980), but this was not reflected in increased duodenal acid levels in the fasting state.

The mean fasting gastric pH of 1.82, with a range of pH 1.3 - 2.4, was similar to that reported previously: a range from pH 1.2 to pH 2.1 (Rhodes, Apsimon and Lawrie 1966, Greenberg et al 1982, O'Sullivan, Harrison and Bullingham 1984, Fimmel et al 1985). In this study, no difference was found between the pH levels of DU patients and healthy subjects, and in both groups, the pH was steady during this period.

Abnormal gastric hypersecretion not only occurs in the basal state, but in some DU patients the hypersecretion occurs at night (Winkelstein 1935, Levin, Kirsner and Palmer 1950), and has been associated with the pathophysiology of duodenal ulcer disease (Baron 1973). This hypersecretion involves the production of abnormally large volumes of gastric juice that contain normal concentrations of acid (Wormsley 1974). This latter observation helps to explain the finding from the

present study that the duodenal bulb exposure to acid at night was not significantly greater than that which occurred during the day. Only at levels of below pH 3 and 2, was this exposure slightly greater. The overall nocturnal pH profile within the bulb was similar to the daytime profile, which like the healthy subjects, followed an exponential curve. Contrary to previous hypotheses, this investigation has shown that the duodenal bulb exposure to acid over the 24 hour period in patients with duodenal ulcer is similar to the exposure experienced by healthy subjects.

Accepting this above finding raises the question of whether the pattern of acid exposure in the bulb is different from that seen in control subjects. The acid peak analysis showed this not to be the case as the overall pattern of acid peaks was similar for DU patients and healthy subjects. However, differences did exist. The DU patients experienced slightly increased exposure during these peaks at night, at the more acidic levels of pH 2 and 3. The similar number of daytime and nocturnal acid peaks suggests that, as in the healthy subjects, the gastric emptying rate is similar during day and night. The rapid and brief fluctuations in acidity appear to be adequately neutralized by duodenal bicarbonate, the secretion of which has been shown to be sufficient in duodenal ulcer patients (Rune and Viskum 1969)*. However, at night, the ratio of short- to long-lived peaks was different from that experienced during the day. There were relatively fewer short-lived peaks. This finding suggests that there is a defect in the duodenal propulsive motility at night in patients with duodenal ulcer. It would appear that this dysmotility is present during the interdigestive phase of gastro-duodenal motor activity. This is supported by evidence presented in an elaborate paper from Japan (Sekiguchi et al 1985).

* This has recently been contested (Isenberg et al 1987: New Engl. J. Med. 316: 374-379).

The daytime intragastric acidity of the DU patients was very similar to that of the controls, with some readings as high as pH 5 - 7. These rises occurred during eating and drinking. The nocturnal profile was more acidic with minimal readings above pH 3.0. Spontaneous rises in pH at night were also demonstrated in the patient group, and presumably relate to duodeno-gastric reflux.

The effect of gastric stimulation (cephalic stimulation and ingestion of a meal) only produced a slight acidic increase in the pH profile in the duodenum, and this effect was similar to that experienced by the controls. Likewise, the pattern of acid peaks was similar to the full daytime pattern and not unlike that of the controls. This confirms earlier reports (Rune and Viskum 1969, Bengtson et al 1986), whereas two further studies (Rhodes and Prestwich 1966, McCloy, Greenberg and Baron 1984) reported significant differences at levels of pH <4 and <2.5 . The rises in gastric pH were to levels in agreement with those previously reported (Fimmel et al 1985), and the return to pre-meal baseline pH values was also similar (Greenberg et al 1982, Rosenkilde-Gram et al 1986).

The findings of this part of the investigation have shown three important facts. Firstly, patients with duodenal ulcer exhibit a duodenal bulb exposure to acid during the full 24 hour period that is similar to that experienced by healthy subjects. Secondly, the pattern of duodenal acidification is similar in DU patients and controls. These two facts suggest that the pathogenesis of duodenal ulcer disease is not primarily related to acid exposure in the duodenal bulb, and that gastric acid plays a secondary role in this disease. The third finding is that

patients with duodenal ulcer exhibit a defect in the duodenal propulsive motility during the interdigestive phase of gastro-duodenal motor activity.

CHAPTER 6

THE ROLE OF HORMONAL CONTROL OF GASTRIC ACID SECRETION IN HEALTH AND DUODENAL ULCER DISEASE

6.1 INTRODUCTION

It has been shown, in the preceding part of this investigation, that the exposure of the duodenal bulb to gastric acid is dependent upon a number of factors. These encompass the rate of gastric acid secretion, the rate of gastric emptying, and the integrity of the local defence mechanisms within the duodenal bulb. These latter defensive factors include the bicarbonate secretion, the state of the duodenal mucosa and the motility of the duodenal bulb. Gastric secretion is controlled and maintained by several interacting forces which may be classified (Hirschowitz 1982) as:

a) neurohumoral: The central figure is the vagus nerve with its secretomotor function. It stimulates gastric secretion and gastric musculature and sphincters by means of transmitters including acetylcholine, bombesin, gastrin and enkephalin, as well as Substance P and vasoactive intestinal peptide (VIP). It operates via the intramural plexuses of Auerbach and Meissener.

b) endocrine: The secretory function of released hormones is stimulatory (gastrin, parathormone via Ca^{++}), inhibitory (secretin, somatostatin, VIP, insulin, ? glucagon) and trophic (gastrin, ? epidermal growth factor).

c) paracrine: These effects are difficult to document and have largely been inferred by the presence of the hormone in gastric juice, or the close proximity of the hormone-producing cells to the parietal cells. Implicated in this role has been gastrin, somatostatin and histamine.

d) mucosal: The bicarbonate ions that are formed in the parietal cells after H^+ secretion, are transported into the interstitial fluid, where they are dependent upon their active removal into the circulation. Reduction in mucosal blood flow causes a reduction in acid secretion.

e) luminal: Factors influencing gastric secretion include the type of food ingested, gastric distension, osmolality of the contents, and the gastric intraluminal pH.

f) cellular: The integrity of the parietal cells, and up-regulation of H_2 and other receptors.

The heterogeneity of duodenal ulcer disease is well-recognised (Malagelada 1979, Rotter 1981, Wormsley 1983, Lam 1984, Lam 1985), and an abnormality of any one, or a combination, of the above factors may be found in patients with duodenal ulcer. These abnormalities may be aetiologically significant.

The third part of this present investigation sought to examine the role played by gastrin, secretin and somatostatin in the physiology and pathophysiology of duodenal bulb acidity. The study protocol, results and discussion are set out in Chapter 7.

6.2 GASTRIN

Early animal experiments regarding the control of gastric acid secretion were performed at the turn of the 20th century, and in 1905, Edkins demonstrated that intravenous injections of antral mucosal extracts stimulated gastric acid secretion in anaesthetized cats (Edkins 1905). He named the active principle "gastrin". However, as the extracts also contained histamine, there followed great controversy as to the presence of gastrin. In 1938, Komarov produced histamine-free extracts that still stimulated acid secretion (Komarov 1938, cited in Ardill 1973), but his methods and results were contested by other workers. "Edkin's hypothesis" was finally proved when, in 1964, Gregory and Tracy, from Liverpool, isolated pure gastrin from hog antral mucosa (Gregory and Tracy 1964). They identified two

heptadecapeptides, gastrin I and II (the difference between them being the presence of a sulfate on the tyrosine of gastrin II). Both gastrins were found to be many times more potent than histamine in stimulating gastric acid secretion. The structures of these peptides were determined (Gregory et al 1964), and they were successfully synthesized (Anderson et al 1964). Two years later, two gastrins (H I and H II) were isolated from human antral mucosa (Gregory and Tracy 1966), their structures determined (Bentley, Kenner and Sheppard 1966) and they were also synthesized (Beacham et al 1966).

Fractionation studies (Yalow and Berson 1970) showed the presence of a larger molecular form of gastrin, which had 34 amino acid residues and was called "big gastrin" (G34). This was found to be the main form of plasma immunoreactive gastrin in patients with pernicious anaemia and Zollinger Ellison (ZE) syndrome. Gregory and Tracy's originally isolated gastrin was referred to as G17, or "little gastrin", and its 17 amino acid sequence of made up the 18-34 sequence of the biologically active C-terminal end of G34. This C-terminal end is a tetrapeptide amide and its sequence is also found in the structurally related, but distinct, hormone cholecystokinin (CCK) (Bryant and Adrian 1982). The third naturally occurring gastrin, G13 or "mini gastrin", has 13 amino acid residues, which correspond to amino acids 22-34 of G34, and 5-17 of G17. (This gastrin has also been referred to as G14 (Bryant and Adrian 1982). An approximately six to eight times greater dose in the molar concentration of G34 than G17 was required to produce the same rate of acid secretion (Walsh and Grossman 1975a), and, despite its lower concentration in the circulation, G17 is probably more important than G34 for stimulation of post-prandial gastric acid secretion (Bryant and Adrian 1982). The gastrin producing cells are the G cells and are distributed from the

gastric corpus to the jejunum. However, the highest concentration of gastrin is in the antrum (predominantly G17, up to 10 nmol/g) and to a lesser extent in the duodenum (predominantly G34) (Malmstrom, Stadil, Rehfeld 1976). The major physiological action of gastrin is the release of gastric acid; the mechanism of which is complex and interrelated with acetylcholine and histamine. The second physiological action is a trophic effect on the gastric mucosa, and to a lesser extent that of the upper small intestine and pancreas. Gastrin also affects most major gastro-intestinal functions, including secretion, absorption and motility.

The release of gastrin is controlled by a combination of a number of factors including peptides and amino acids, gastric distension, vagal stimulation (via bombesin), and to a far smaller extent, blood-borne calcium and adrenaline (Walsh and Grossman 1975a, Richardson et al 1977, Hirschowitz 1982). Meals rich in protein produce larger rises in gastrin than those meals rich in carbohydrate or fat. The vagal effect is mainly seen during the cephalic phase of gastric secretion and after gastric distention, and is also elicited by insulin hypoglycaemia (Hansky et al 1971). In the cat and dog, vagally-released gastrin is required for effective direct vagal stimulation of the parietal cells. In man, however, this interaction is not as important. During the interdigestive phases, the gastrin levels return to basal values (Jorde and Burhol 1985). No diurnal variation has been noted (Moore and Wolfe 1973, Ellis et al 1975). A negative feedback loop modulates the release of gastrin; the gastric acid secreted in response to gastrin release reaches the antrum and inhibits further gastrin secretion (Fe Thein and Schofield 1959). The threshold pH is believed to be pH 2.5 (Nilsson 1979). The presence of acid also inhibits all stimulants of gastrin release, as well as gastrin itself either by direct interaction

with the open end of the G cell or by the release of somatostatin (Hirschowitz 1982). The vagus, via somatostatin (Reichlin 1986), acts to inhibit gastrin secretion. In addition, the endogenous peptides, secretin, glucagon, vasoactive intestinal peptide and gastric inhibitory peptide, act to inhibit the release of gastrin from the G cells, but their capability to do so at their normal plasma concentrations has been doubted (Walsh and Grossman 1975b).

6.3 SECRETIN

Evidence for pancreatic secretion to be stimulated by the presence of "acid chyme" in the duodenum inspired Bayliss and Starling to investigate the mechanism of such secretion. They demonstrated that acidification of denervated small bowel caused the prompt secretion of alkaline pancreatic juice. Their studies led to the discovery of secretin (Bayliss and Starling 1902), which they proposed to be a blood-borne messenger in being absorbed from the duodenal mucosa into the circulation and carried to the pancreas where it stimulated the secretion of pancreatic juice, rich in bicarbonate. They called it a "hormone", a term which is derived from the Greek for "I arouse to activity". Their paper was the first to propose the chemical co-ordination of bodily functions, and was the beginnings of endocrinology.

The structure of secretin is that of a polypeptide chain of 27 amino acids, all of which are necessary for biological activity. Great similarities in structure have been reported between secretin and glucagon, gastric inhibitory polypeptide and vasoactive intestinal polypeptide such that these four polypeptides may be considered as one hormonal family (Greenberg 1982). Secretin is localized to the S cell

which is found in highest concentration in the duodenal mucosa and somewhat smaller amounts in the jejunum (Bloom and Polak 1980).

The release of secretin is stimulated by acid in the duodenum (Ward and Bloom 1975, Kurokawa et al 1982); the threshold pH of which is recognised to be pH 4.0 or less (Rhodes and Prestwich 1966, Dalton et al 1976, Pelletier et al 1978). The responses to duodenal acidification, although significant, are small and transient (Pelletier et al 1978, Greenberg et al 1982) but are clearly detected by the recently improved radioimmunological techniques which yield greater assay sensitivity. Inhibition of secretin release is produced directly by somatostatin (Boden et al 1975, Wolfe, Reel and McGuigan 1983), and indirectly by gastric acid suppression by H_2 antagonists. Although the main effect of secretin is stimulation of pancreatic secretion of bicarbonate, an effect potentiated by CCK, its gastric effect is to inhibit the acid-producing effect of gastrin (Wormsley and Grossman 1964, Johnson and Grossman 1971). The action is one of non-competitive inhibition, as secretin acts on a different receptor site compared to gastrin (Johnson and Grossman 1971).

6.4 SOMATOSTATIN

Somatostatin was first isolated in 1973 by Brazeau et al from the bovine hypothalamus (Brazeau et al 1973), and has since been identified in many mammalian tissues especially brain, gut (highest concentration in antrum) and pancreas. It is predominantly found in neurones throughout the brain and spinal cord and in the endocrine D cells of the gut mucosal layer, and at the periphery of the islets of Langerhans in the pancreas (Lucey 1986). In the foetal and neonatal pancreas, it is the second most abundant hormone after insulin

(O'Shaughnessy 1982). Its fourteen amino acids are held in a ring cystine bridge - Somatostatin-14. A larger precursor form has been identified: Somatostatin-28 with twenty eight amino acids (Lucey 1986). Apart from its neurotransmitting and neurohumeral regulatory actions, it has powerful inhibitory actions on the gut, pancreas and gastro-intestinal hormones.

Somatostatin is released into the peripheral circulation following the ingestion of food, especially fat and protein (Lucey et al 1984). The importance of the vagus in mediating circulating somatostatin release is evidenced by the releasing effects of insulin hypoglycaemia and atropine. Post-prandially, the levels return to basal values (Jorde and Burhol 1985). The vagus in man has an important control in the circulating somatostatin release. Gastric acid does not appear to be a major stimulant (Lucey 1986). This latter finding contrasts the response in dogs. Animal studies have demonstrated direct secretion of the hormone into the gut lumen, and this, together with the close proximity of the D and G cells, suggests a paracrine effect.

The release of all gut peptides are inhibited by somatostatin, and it appears to regulate gastric acid secretion by both paracrine and endocrine effects (Lucey 1986). It has been suggested that somatostatin is an "enterogastrone" - a circulating hormone released by intraduodenal fat which inhibits acid secretion. In addition, somatostatin and gastrin are thought to be linked within the stomach as part of a local negative feedback loop interacting via the vagus (Reichlin 1986).

6.5 RADIOIMMUNOASSAY TECHNIQUES

The principle of radioimmunoassay is one of competition between the radio-labelled and unlabelled hormone for specific binding sites of an antibody. The higher the concentration of the unlabelled hormone, the smaller the amount of radio-labelled hormone is bound to the antibody. As the amounts of radio-labelled hormone and antibody are fixed and known, the concentration of the unlabelled hormone sample may be calculated indirectly by separating the bound from free hormone and counting the radioactivity of the bound portion. Standard curves are prepared by using known concentrations of pure hormone, and these are compared with the unknown hormone sample. Ideally, the standards should be prepared from hormones of the same species as the sample, as significant differences occur between species. In some cases, porcine hormones are used due to the lack of isolated human hormones (eg. secretin). The sensitivity of the assay increases with the increasing steepness of the standard curve and high reproducibility, thus detecting small differences in concentrations of samples. A longer incubation period, up to a maximum of six days, increases the assay sensitivity and this is used when measuring hormones of low plasma concentrations. The levels detected by radioimmunoassay are far smaller than those detected by most bioassays. The optimum temperature for performing the assay is 4°C, as at this temperature, bacterial contamination and proteolytic degradation is minimized. Three main methods of separation of bound from free hormone exist: immunoprecipitation, chemical precipitation and absorption. Charcoal is used commonly in the latter method as it is simple, rapid and cheap. The addition of dextran to the charcoal prevents absorption of the antibody complex.

In the following chapter, the interrelationship of these three hormones to intraluminal pH and their responses to feeding are investigated.

CHAPTER 7

EFFECT OF MODIFIED SHAM FEEDING AND INGESTION OF A
SOLID MEAL ON PLASMA GASTRIN, SECRETIN AND
SOMATOSTATIN AND CONCURRENT CHANGES IN DUODENAL
BULB pH IN HEALTHY SUBJECTS AND PATIENTS WITH
DUODENAL ULCER

7.1 HYPOTHESIS II

Antral pH regulates gastric acid secretion by activation of neural and hormonal mechanisms. Vagal stimulation causes the secretion of gastric acid by a direct effect on the parietal cells and an indirect effect by stimulating the production of gastrin. The resultant acid pH in the gastric antrum inhibits both further gastric acid secretion and gastrin release (Woodward et al 1954, Walsh, Richardson and Fordtran 1975, Fiddian-Green and Vinik 1976, Befrits, Samuelsson and Johansson 1984). In addition, both gastrin and gastric acid secretion are inhibited by somatostatin (Johnson and Grossman 1971), the release of which is stimulated by the vagus (Lucey et al 1985). The inhibition via somatostatin is diminished in patients with duodenal ulcer (Harty, Maico and McGuigan 1986). It has been postulated that the defect in somatostatin inhibition is responsible for the acid hypersecretion and post-prandial hypergastrinaemia seen in duodenal ulcer patients (Stadil et al 1975, Calabro et al 1986).

It is well established in dogs that acidification of the duodenum causes inhibition of gastric acid secretion (Uvnas 1971, Lanas et al 1985). This negative feedback also occurs in man (Shay et al 1942, Johnston and Duthie 1964, Wormsley and Grossman 1964, Bochenek, Rodgers and Balint 1971) but the response is variable (Wormsley 1974, Sainz et al 1985). It has been suggested that this inhibition by duodenal acidification is defective in duodenal ulcer patients (Johnston and Duthie 1964, Malagelada et al 1977). The process of inhibition is thought to be humoral via the release of secretin which then inhibits the gastric acid secretion and further gastrin release (Bloom and Ward 1975, Sainz et al 1985). It has been postulated that the release

of endogenous secretin is diminished in patients with duodenal ulcer (Fiddian-Green and Hobsley 1974, Bloom and Ward 1975, Kurokawa et al 1982, Sainz et al 1985).

7.2 STUDY AIM

In order to examine the above hypotheses, this study (Study III) was set out to assess the effect of the cephalic and gastric phases of gastric secretion on plasma gastrin, secretin and somatostatin and duodenal bulb pH in patients with active duodenal ulcer and healthy subjects. The interrelationship of the three hormones to one another was also investigated.

7.3 STUDY GROUPS

Group 1: Normal healthy subjects (n=20). This group consisted of most of the subjects from Study I of this investigation (Chapter 4) plus two additional subjects. The same criteria for inclusion and exclusion applied. There were 13 males and 7 females, and the mean age was 25.2 years (range 19-67). The oldest subject, FA, from Study I was not included in this third study.

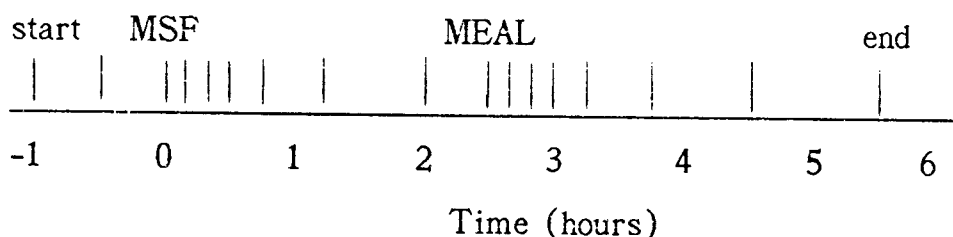
Group 2: Duodenal ulcer patients (n=22). These patients formed a subgroup of those studied in Study II (Chapter 5). The criteria for inclusion and exclusion were the same. The discrepancy in numbers between Studies II and III is due to the fact that Study III was commenced later. Patient A.R., who had previously undergone a highly selective vagotomy for duodenal ulcer disease and presented with a recurrent ulcer, was also investigated in this study. His results, for comparative purposes, are reported separately.

The mean age of the patient group was 43.5 years (range 23 - 72).

7.4 STUDY DESIGN (Figure 7.1)

This study lasted six and a half hours. The design was similar to that described in Study I. The dual pH monitoring system was used in similar fashion, and the study period incorporated a fasting period, cephalic stimulation by modified sham feeding and the ingestion of a solid meal. The same two meals (Illustrations 4.1 and 4.2) for the modified sham feeding and the ingested solid meal were served. The duration of the modified sham feed was thirty minutes.

Figure 7.1: Study design - Study III: The arrows indicate the time of venous blood sampling (see text)



At the start of the study, an 18G Venflon^R catheter was inserted into a vein in the ante-cubital fossa for repeated blood sampling. The catheter did not required to be heparinised nor flushed with saline before sampling. Serial blood samples were taken at times as indicated in Figure 7.1; at 60, 30 and 0 minutes before, every 10 minutes during, and at 15, 30, 45 and 60 minutes after first cephalic stimulation by modified sham feeding, and the ingestion of the standardised solid meal.

The blood samples were collected in heparinised tubes on ice, centrifuged as soon as possible at 2500 rpm and 4°C for 15 minutes and the plasma was then pipetted into a test tube containing 1000 U of Trasylol^R. The sample was stored at -20°C until assayed.

The radioimmunoassays for gastrin, secretin and somatostatin were performed in the Department of Medicine (Professor K.D. Buchanan), Queen's Hospital of Belfast, Belfast, Northern Ireland. The plasma samples were packed frozen into a polystyrene box, filled with dry ice, and transported by courier post to Belfast. The transit time was short, being approximately sixteen to eighteen hours, and prevented thawing of the samples before assaying was ready.

7.4.1 Plasma Gastrin Radioimmunoassay

An antibody was raised in rabbits against synthetic human gastrin I (ICI), and this was used in the assay, 0098, at an approximate final dilution of 1:10 000. This antibody did not cross-react with other gastro-intestinal peptides except cholecystokinin-pancreozymin (CCK), but greater than 10 000 times concentration of CCK was required to produce a similar fall in bound counts as did standard gastrin. The antibody also recognized Big gastrin (G34). The standards used for the assay were gastrin human-type synthetic 68/439. Hormone-free human plasma, separated by charcoal, was added to the standards so as to equilibrate the conditions between plasma samples and standards. The radioimmunoassay (Ardill 1973) was then performed.

7.4.2 Plasma Secretin Radioimmunoassay

Plasma for the secretin assay was extracted using ethanol. Pork synthetic secretin (SG 18773), used for the standards, was labelled with ¹²⁵I and antibodies were raised against bovine natural secretin.

The antibody, BB101, was used at a final titre of 1:36 000 and was specific for secretin, as no cross-reactivity was observed with other peptides (pancreatic glucagon, insulin, gastric inhibitory polypeptide, motilin, vasoactive intestinal polypeptide, human synthetic gastrin and 99% pure cholecystokinin-pancreozymin). The sensitivity of the assay was established at 6 ng/l with a highly purified ^{125}I secretin.

7.4.3 Plasma Somatostatin Radioimmunoassay

The frozen plasma samples were reconstituted prior to the assay in 0.04 M phosphate buffer of pH 7.4. Synthetic cyclic somatostatin was used as the standard, to which the antibody, OB 5(1), was raised. This antibody was then used in a final dilution of 1:15 000 for the assay. There was no cross-reactivity with any other gut peptides. N-tyrosylated somatostatin was first iodinated then purified on a cation exchanger, Whatman CM52, using 0.05 M and 0.25 M acetate buffers. In order to equilibrate conditions between the standards and plasma samples, horse serum was first charcoaled to remove endogenous peptides, extracted by alcohol, then added to the calibration samples. The separation of the antibody-bound peptide from free peptide was achieved using dextran-coated charcoal. The sensitivity of the assay was 3 ng/l (95% confidence).

7.5 ETHICAL APPROVAL

The protocol of Study III was approved by the Ethics Committee of the University of Dundee, and the Tayside Health Board.

7.6 RESULTS

7.6.1 Plasma Gastrin

The control group of healthy subjects exhibited a median fasting plasma gastrin level of 20.0 ng/l, (range 5.0 - 110.0 ng/l). This level was fairly constant, as demonstrated by the median levels taken during the fasting period (at -60, -30, and 0 minutes): 22.5, 20.0 and 25.0 ng/l. The values of medians and ranges were used due to the skewness of the data. As four extreme values existed, the sample mean of the fasting gastrin data was calculated at 29.3 ng/l with 1 standard deviation of 22.03. This data was skewed to the right, and the test of skewness gave a value of +2.06; (data of the ideal normal distribution has a skewness value of 0.00, while a skew to the right (positive) has an upper tail greater than the lower tail, and a skew to the left a lower tail greater than the upper tail). The distribution of the fasting gastrin data is illustrated in Figure 7.2.

In order to normalize the distribution, this data was transformed by natural logarithms. The geometric mean of the fasting plasma gastrin level of the control group was 23.8 ng/l, and the skewness value 0.09. (The sample median using this transformation was 19.99 ng/l.) Thus, the fasting plasma gastrin data of the healthy subjects showed a log-normal distribution.

The fasting plasma gastrin level of the patients with duodenal ulcer had a median value of 55.0 ng/l and a range of individual values from 20.0 to 105.0 ng/l. The level was also fairly constant; the three median fasting values being 65.0, 55.0 and 50.0 ng/l. This fasting level was significantly higher than that of the healthy subjects ($p < 0.01$, Mann-Whitney U Test).

FASTING PLASMA GASTRIN LEVELS IN DU PATIENTS AND CONTROLS

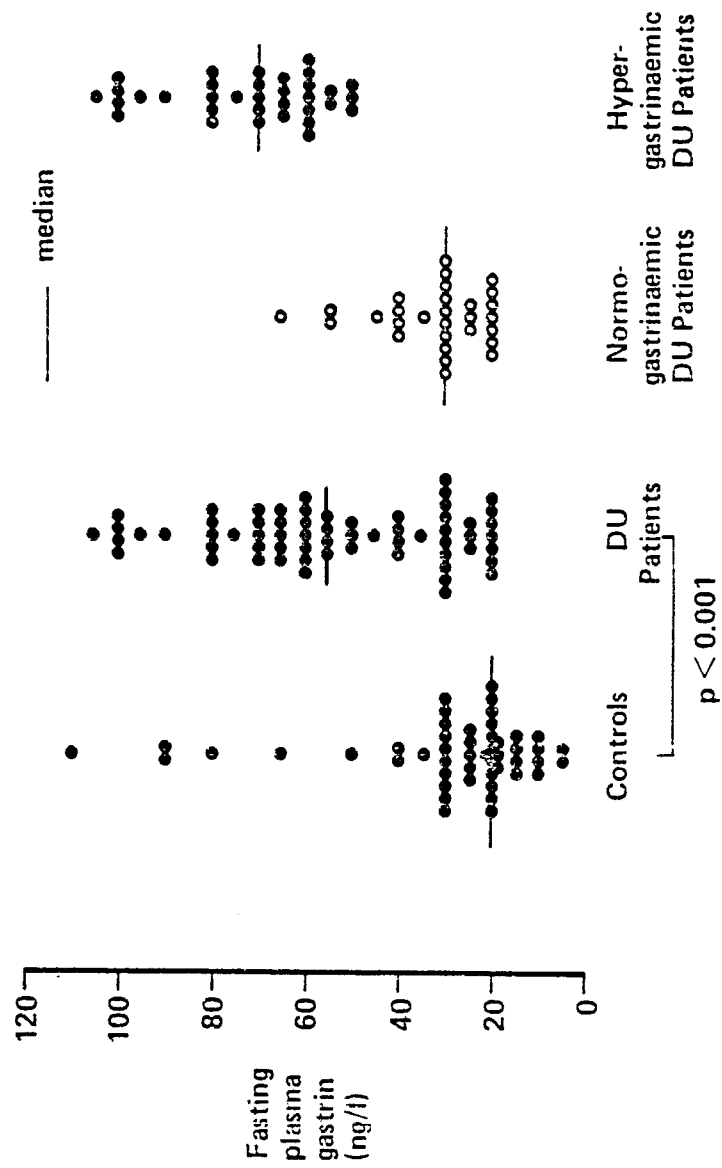


Figure 7.2

Whilst the control subjects displayed data skewed to the right, the DU patients exhibited a more normal distribution of fasting gastrin data with a skewness value of +0.30. However, the pattern showed a definite bimodal distribution (discussed below), as can be seen in Figure 7.2. The data was also transformed by natural logarithms so as to further normalize the distribution and this yielded a geometric mean of the patient data of 54.99 ng/l. The skewness of the transformed data was similar at -0.35.

At all times during the 6.5 hour study, the DU patient group exhibited significantly higher plasma gastrin levels compared to the controls (Figure 7.3). Cephalic stimulation by modified sham feeding (MSF) produced a negligible increase in plasma gastrin of the healthy subjects, to a median level of 25.0 ng/l (range 5.0 - 120.0) after 10 minutes. This stabilized at 22.5 ng/l (range 5.0 - 140.0) after 30 minutes and represented an increase over fasting levels of 12.5%. The response of the DU patients to cephalic stimulation was greater, and it caused the plasma gastrin level to rise to a median peak value of 70.0 ng/l, (range 20.0 - 200.0), after 30 minutes. This was an increase of 27.3% over fasting levels but failed to reach statistical significance. This peak level was, however, significantly higher than the peak of the healthy subjects ($p < 0.001$, Mann-Whitney U test).

During the two hours following completion of the modified sham feed, the median plasma gastrin level of the controls did not show any appreciable fluctuation. The gastrin levels of the DU patients remained somewhat elevated and decreased to a median value of 40.0 ng/l prior to the start of the second feed. This final level was still significantly greater than that of the controls ($p < 0.01$).

EFFECT OF MODIFIED SHAM FEEDING (MSF) AND A MEAL ON PLASMA GASTRIN
IN DUODENAL ULCER PATIENTS AND HEALTHY SUBJECTS

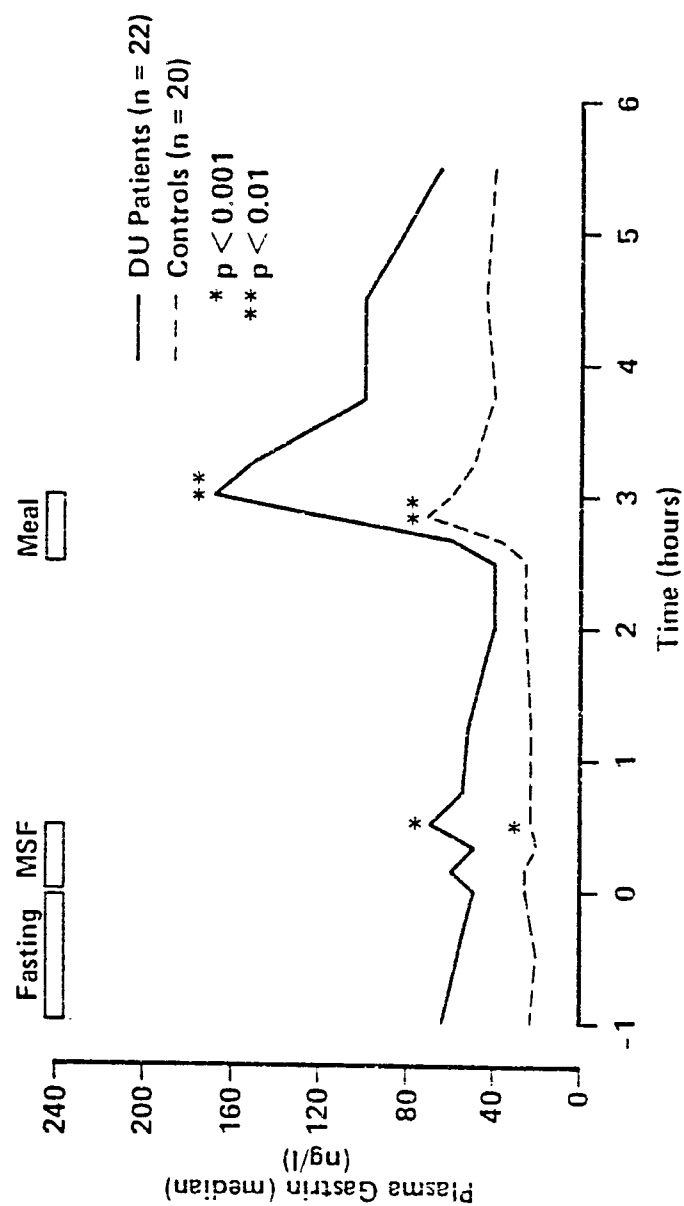


Figure 7.3

The ingestion of a solid meal caused an increase in plasma gastrin amongst the healthy subjects to a median peak value after 20 minutes of 70.0 ng/l (range 20.0 - 300.0). This represented a significant increase over pre-meal values of 180.0% ($p < 0.001$, Wilcoxon Matched-Pairs Signed-Ranks test). The patients with duodenal ulcer responded in a significantly greater fashion. The plasma gastrin level increased sharply to a median peak value after 30 minutes of 167.5 ng/l (range 20.0 - 325.0). This was a significant 318.8% increase over pre-meal levels ($p < 0.001$). In both study groups, the meal peak was significantly higher than the peak following cephalic stimulation ($p < 0.001$, Wilcoxon Matched-Pairs Signed-Ranks test).

The plasma gastrin levels of the controls decreased initially during the post-prandial period but remained slightly elevated until the end of the study. The DU patients demonstrated a more prolonged elevation in plasma gastrin, and at three hours after the meal, the levels were still raised compared to pre-meal values.

Closer inspection of the patient data revealed two separate populations of duodenal ulcer patients. As noted above, the fasting gastrin data showed a bimodal distribution (Figure 7.2) with those patients in the high group showing minimal overlap with those in the low group. On the basis of this data, the DU patient group was divided into two sub-groups. The high group ($n=11$), due to their exaggerated gastrin levels, were termed the "Hypergastrinaemic" (H^{RG}) patients. The low group ($n=11$) displayed gastrin levels similar to the healthy subjects and were termed the "Normogastrinaemic" (N^{OG}) patients. As shown in Figure 7.2, the median fasting plasma gastrin level of the H^{RG} DU

patients was 70.0 ng/l (range 50.0 - 105.0) compared to a median fasting level of 30.0 ng/l (range 20.0 - 65.0) in the N^OG DU patients.

Table 7.1 shows the fasting gastrin levels in the Hypergastrinaemic and Normogastrinaemic DU patients and healthy subjects, as well as the respective responses to cephalic stimulation and ingestion of the solid meal.

Table 7.1: Plasma gastrin responses to cephalic stimulation (MSF) and a meal in "Hypergastrinaemic" (H^RG) and "Normogastrinaemic" (N^OG) DU patients and healthy subjects (VOL).

PLASMA GASTRIN (ng/l) [median(range)]					
	Fasting	MSF Peak	Meal Peak	p value*	
				F/MSF	MSF/M
H ^R G	70.0	90.0	225.0	<0.02	<0.001
(n=11)	(50-105)	(70-200)	(150-325)		
N ^O G	30.0	35.0	92.5	NS	<0.001
(n=11)	(20-65)	(20-60)	(20-175)		
VOL	20.0	22.5	70.0	NS	<0.001
(n=20)	(5-110)	(5-140)	(20-300)		

*Wilcoxon Matched-Pairs Signed-Ranks Test for Fasting vs MSF, MSF vs Meal.

Only the Hypergastrinaemic patients demonstrated a significant rise in plasma gastrin over fasting levels following cephalic stimulation. Thirty minutes after ingestion of the meal, the H^RG patients experienced an exaggerated gastrin response to a median peak value of 225.0 ng/l (range 150.0 - 325.0). This was an increase of 309.1% over

the pre-meal value. Thereafter, the gastrin level dropped sharply then plateaued to remain elevated until the end of the study. The Normogastrinaemic patients exhibited a median peak gastrin level, 45 minutes after the meal, of 92.5 ng/l (range 20.0 - 175.0). This was a 208.3% increase over pre-meal values. This group of patients also showed sustained elevated levels of gastrin in the post-prandial period.

The differences in responses are more readily appreciated when illustrated graphically and are shown in Figure 7.4. The full plasma gastrin data for the DU patients as a group, the H^RG and N^OG subsets and healthy subjects are shown in the Appendix in Tables A.5 and A.6.

Patient A.R. (previous HSV, and recurrent ulcer) showed a mean fasting plasma gastrin level of 120 ng/l. This level rose to a peak of 150 ng/l twenty minutes after the sham feed, and returned to basal levels in one hour. The response to the ingested meal was greater than any of the other patients studied, and reached the peak level of 385 ng/l twenty minutes after the meal. At the end of the study, the plasma gastrin level remained elevated at 250 ng/l.

7.6.2 Plasma Secretin

The median fasting plasma secretin level in the healthy subjects was 25.0 ng/l and this ranged from 5.0 to 55.0 ng/l. This level remained constant throughout the fasting period. The data distribution was more normal (skewness value 0.47); (the mean value was 24.89 ng/l, SD 11.16). Transformation of the data by natural logarithms did not provide any advantage to the distribution. The patients with duodenal ulcer, as a group, exhibited the median fasting plasma secretin level of 25.0 ng/l, and the range was wider than the controls at 0.0 - 75.0

EFFECT OF MODIFIED SHAM FEEDING (MSF) AND A MEAL ON PLASMA GASTRIN IN
HYPERGASTRINAEMIC (HrG) AND NORMOGASTRINAEMIC (NoG) DU PATIENTS AND CONTROLS

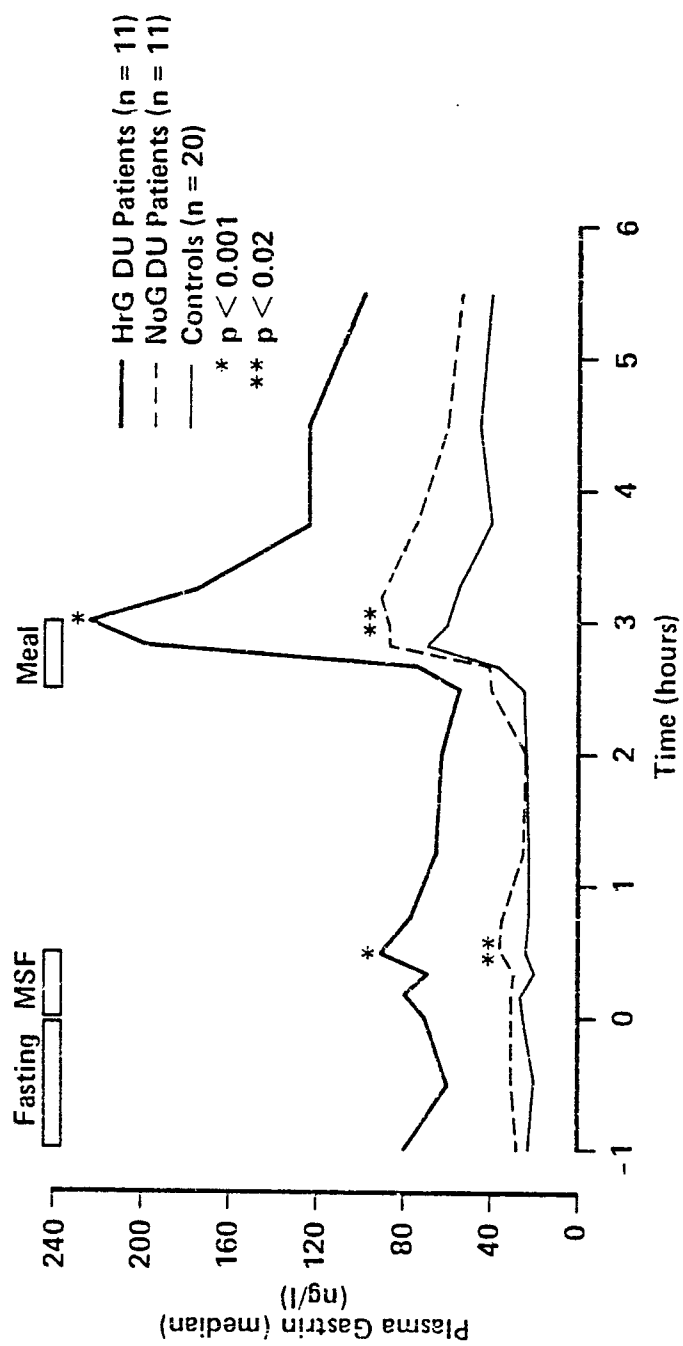


Figure 7.4

ng/l. The distribution was skewed slightly to the left (value -0.91), and, as with the controls, transformation by natural logarithms was not beneficial. Comparison of the fasting levels of both study groups showed no statistical difference.

Figure 7.5 shows the responses of plasma secretin to cephalic stimulation and ingestion of a meal in the DU patients and controls. Cephalic stimulation produced a small decrease in plasma secretin after 30 minutes, among the healthy subjects and this returned to the fasting level fifteen minutes later. The DU patients experienced an initial slight fall in plasma secretin which, after returning to the fasting level, was followed by a rise of the same magnitude fifteen minutes after stimulation. During this period of stimulation, there was no statistical difference between the groups. The ingestion of a meal caused a decrease in plasma secretin after ten minutes in the controls, and this dropped to a minimum level of 17.5 ng/l (range 5.0 - 35.0) 75 minutes after the start of the meal. The decreased level was prolonged to the end of the study. The patients with duodenal ulcer showed a smaller decrease in plasma secretin twenty minutes after eating which was less prolonged than in the controls. There were no significant differences in the plasma secretin levels between the two groups. The data is presented in Table A.7.

Using the fasting gastrin data to divide the DU patients into the two previously defined groups, the secretin data was re-examined. The distributions of the fasting secretin values were not improved by log-transformation. Figure 7.6 shows the plasma secretin responses to the cephalic stimulation and ingestion of the meal in the "Hypergastrinaemic" and "Normogastrinaemic" patients and the cohort of healthy subjects. The H^RG ulcer patients exhibited slightly higher

EFFECT OF MODIFIED SHAM FEEDING (MSF) AND A MEAL ON PLASMA SECRETIN
IN DUODENAL ULCER PATIENTS AND HEALTHY SUBJECTS

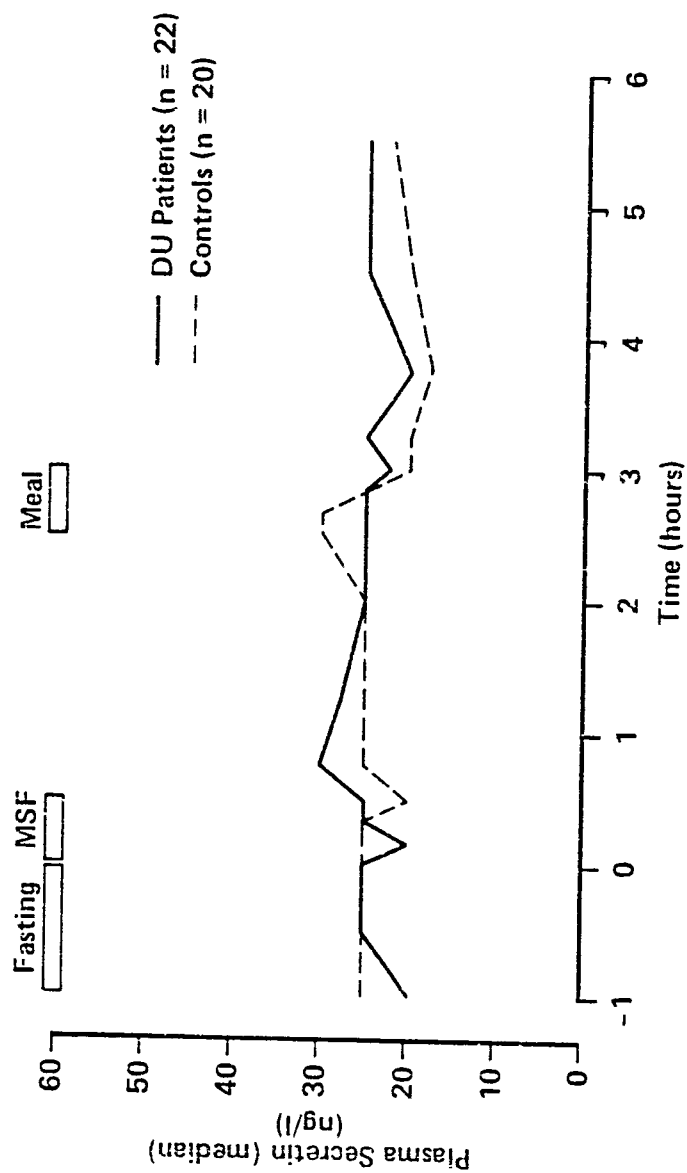


Figure 7.5

EFFECT OF MODIFIED SHAM FEEDING (MSF) AND A MEAL ON PLASMA SECRETIN IN
HYPERGASTRINAEMIC (HrG) AND NORMOGASTRINAEMIC (NoG) DU PATIENTS AND CONTROLS

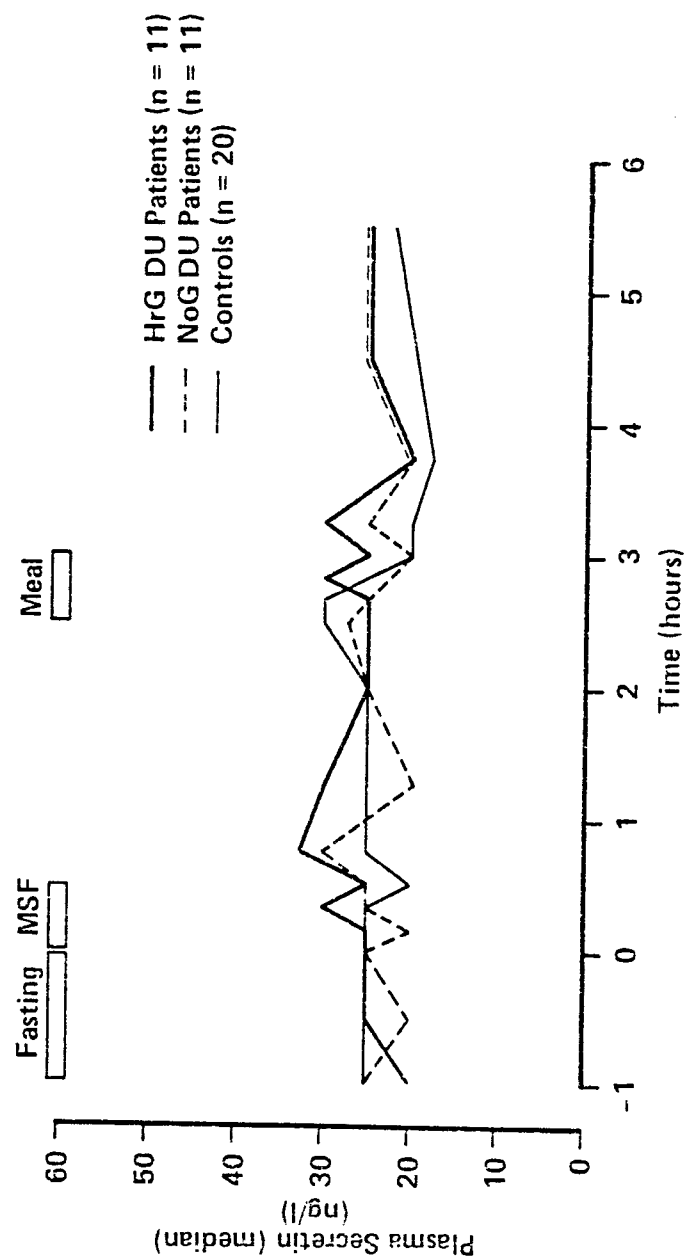


Figure 7.6

median fasting levels compared to the N^OG group: 25.0 ng/l (range 10.0 - 75.0) versus 22.5 ng/l (range 0.0 - 50.0); these levels were not significantly different. Following cephalic stimulation, the Hypergastrinaemic patients demonstrated a small rise in plasma secretin which was followed by a larger rise at fifteen minutes after completion of the sham feed. The return to fasting levels occurred after 75 minutes. The Normogastrinaemic patients experienced an initial decrease in plasma secretin and a subsequent rise co-inciding with that of the H^RG patients.

The ingestion of the meal produced an initial increase in the plasma secretin levels of the H^RG patients, while those of the N^OG group decreased. Thereafter, there was no difference between the responses of the two groups. At no time during the study, were the differences in plasma secretin responses between the three groups (H^RG, N^OG, Controls) statistically significant. The full data of the plasma secretin responses are shown in the Appendix in Table A.8.

Patient A.R. showed a mean fasting secretin level of 11.7 ng/l, and a small peak at twenty minutes after MSF of 20.0 ng/l. He showed no response to ingestion of the meal as the secretin level remained at 10.0 ng/l.

7.6.3 Plasma Somatostatin

The median fasting plasma somatostatin level of the healthy subjects was 10.0 ng/l, and this ranged widely from 1.0 to 105.0 ng/l. The median values obtained during the fasting period showed minimal variation (10.0, 10.0 and 9.0 ng/l). As with the gastrin data, the distribution was skewed to the right and showed a skewness value of +2.25. Transformation of this data by natural logarithms improved the

distribution to near-normal (skewness value of +0.18). The geometric mean was calculated to be 10.28 ng/l (SD 2.75, SEM 1.15). As a group, the patients with duodenal ulcer demonstrated a higher median fasting plasma somatostatin level at 15.0 ng/l, and the range of values was similar to the controls being from 1.0 to 90.0 ng/l. The difference between the fasting levels of the two groups was significant ($p < 0.01$, Mann-Whitney U-test). Like the controls, there was minimal variation in the three fasting values. The distribution of the patient data was not normal (skewness value +1.15). Logarithmic transformation improved the distribution to more normal (value -0.37), and the geometric mean of this data was 16.45 ng/l (SD 2.44, SEM 1.12).

Figure 7.7 shows the plasma somatostatin responses to the cephalic stimulation and ingestion of the meal in the DU patients as a group and the healthy subjects. There was no appreciable change in the plasma somatostatin level of the healthy subjects after cephalic stimulation, although some fluctuation was observed. The DU patients exhibited a definite response at ten minutes after stimulation and a median peak value of 20.0 ng/l (range 2.0 - 125.0) was measured. The difference between the two study groups remained significant at $p < 0.05$ level. A similar response was observed after the meal. The controls experienced a short-lived drop in plasma somatostatin at twenty minutes after the start of the meal, whilst the DU patients showed a second 33.3% increase to 15.5 ng/l. The post-prandial somatostatin plasma levels were similar for the two groups. Table A.9 shows these results in tabular form.

The two sub-sets of duodenal ulcer patients showed markedly different plasma somatostatin responses. The Hypergastrinaemic

EFFECT OF MODIFIED SHAM FEEDING (MSF) AND A MEAL ON PLASMA SOMATOSTATIN IN DUODENAL ULCER PATIENTS (n = 22) AND HEALTHY SUBJECTS (n = 20)

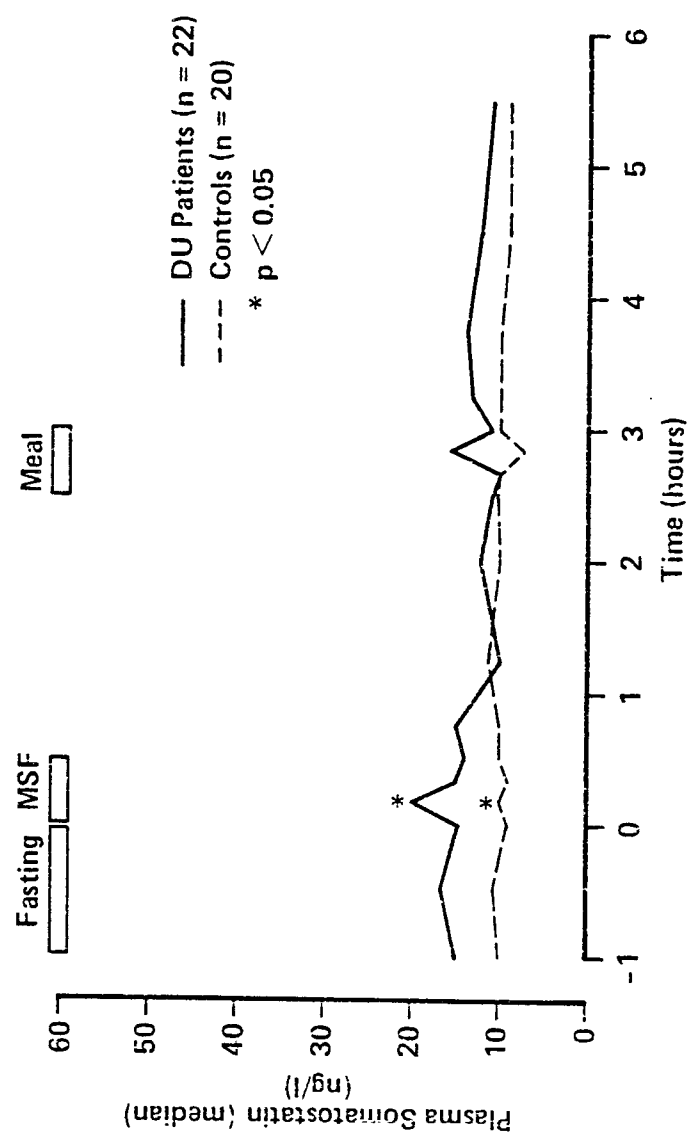


Figure 7.7

EFFECT OF MODIFIED SHAM FEEDING (MSF) AND A MEAL ON PLASMA SOMATOSTATIN
IN HYPERGASTRINAEMIC (HrG) AND NORMOGASTRINAEMIC (NoG) DU PATIENTS AND CONTROLS

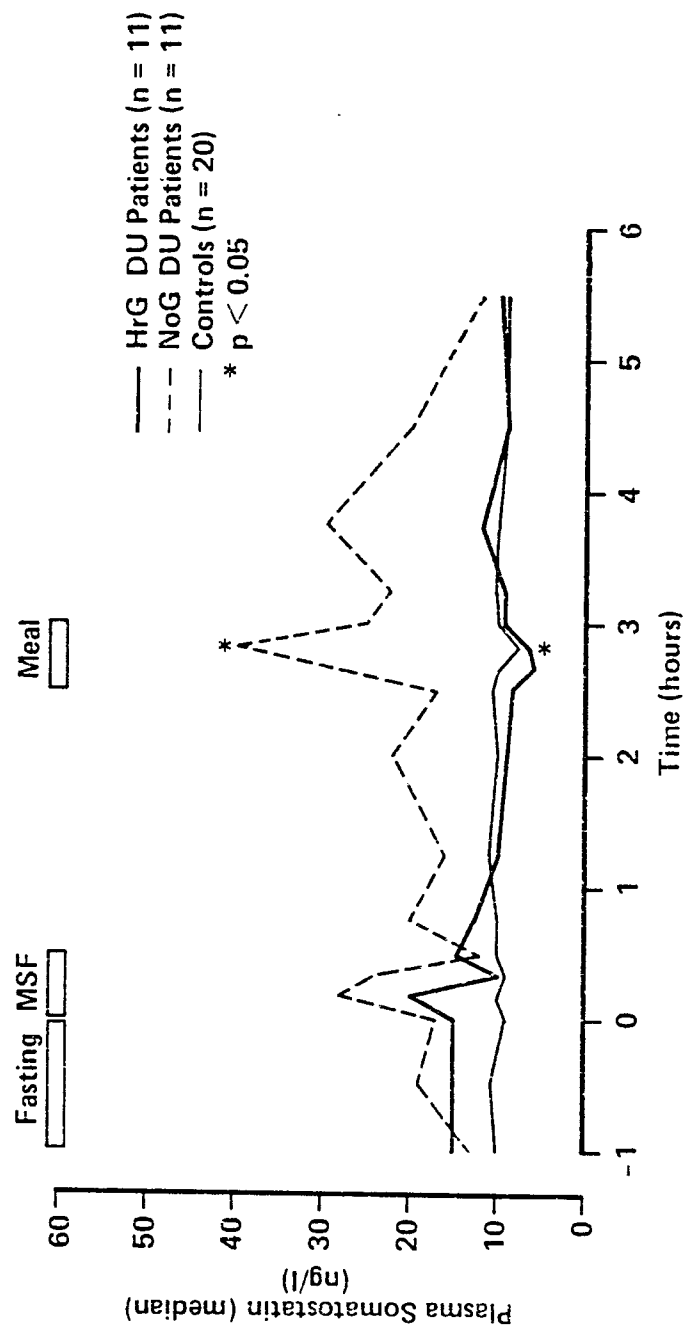


Figure 7.8

patients (Figure 7.8) showed a lower median fasting plasma somatostatin level than that of the Normogastrinaemic patients; the difference being significant ($p < 0.05$, Mann-Whitney U test). Compared to the control group, the fasting level of the N^OG patients was significantly higher ($p < 0.01$). The response to cephalic stimulation was similar for both H^RG and N^OG patient groups but the latter group experienced a stronger response. The median peak value after ten minutes of cephalic stimulation of the N^OG patients was 28.0 ng/l (range 5.0 - 65.0) and this was significantly higher ($p < 0.05$) than that of the H^RG patients (median 20.0 ng/l, range 2.0 - 125.0), as well as the controls ($p < 0.01$). Following stimulation, the N^OG patients exhibited plasma somatostatin levels similar to the fasting levels, whereas the H^RG patients experienced a decrease in the levels.

Following the ingestion of the meal, the Normogastrinaemic patients demonstrated an immediate increase in plasma somatostatin to a median peak value of 40.0 ng/l (range 5.0 - 60.0) at twenty minutes. The post-prandial period was characterized by a persistently elevated plasma somatostatin level, punctuated by a second rise 45 minutes after completion of the meal. The Hypergastrinaemic patients showed a quite different response which was very similar to that of the healthy subjects. The tabular format of this data is presented in Table A.10.

Patient A.R. showed minimal plasma somatostatin responses to stimulation. The fasting level was 8.7 ng/l, and cephalic stimulation produced a decrease in the level to a low of 3.0 ng/l. Likewise, the meal caused a fall in plasma somatostatin levels to 1.0 ng/l which later returned to basal levels.

7.6.4 Correlation between these hormones

Both secretin and somatostatin produce an inhibition of gastrin release. In dogs, a positive correlation between secretin and gastrin levels has been demonstrated, but in man, similar evidence is lacking. Gustavsson and co-workers found no correlation between gastrin and somatostatin levels in ulcer patients, but only fasting levels were investigated (Gustavsson et al 1982). In order to test the hypotheses, the correlation between post-stimulation plasma gastrin and plasma secretin levels, and plasma gastrin and plasma somatostatin levels were calculated from the results of this investigation.

i) Gastrin : Secretin

Following feeding stimulation (modified sham feeding and ingestion of the meal), the healthy subjects exhibited a significantly negative correlation between plasma gastrin and plasma secretin levels, (Spearman's rank correlation coefficient $r_s = -0.218$, t value = -3.555 with 254 degrees of freedom [d.o.f.], $p < 0.001$). Thus, an inverse relationship between plasma gastrin and secretin appeared to exist amongst the controls. The distribution of data, however, is variable, with the gastrin data being negatively skewed and the secretin data being near-normally distributed. For greater accuracy, the log-transformed plasma gastrin data and the original plasma secretin data were used for the analysis by simple linear regression. The correlation between the log-gastrin and secretin appears to be a linear one, with the slope of the line being -3.69 ($p < 0.001$).

The Hypergastrinaemic patients showed no significant correlation between the plasma gastrin and plasma secretin levels, (Spearman's rank correlation coefficient $r_s = -0.049$, t value = -0.594 with 147 d.o.f., $p = \text{NS}$). Although statistical significance was not reached, the

relationship between these two hormones was inversely inclined. The Normogastrinaemic patients, on the other hand, displayed a highly significant negative correlation between plasma gastrin and plasma secretin levels (Spearman's rank correlation coefficient $r_s = -0.332$, t value = -4.116 with 137 d.o.f., $p < 0.001$). After transforming this latter gastrin data by natural logarithms, a simple linear regression appears to exist with the slope of the line -0.41 ($p < 0.001$).

ii) Gastrin : Somatostatin

The healthy subjects demonstrated no significant correlation between these two hormones (Spearman's rank correlation coefficient $r_s = 0.033$, t value 0.537 with 262 d.o.f., $p = \text{NS}$). The correlation did, however, appear to be positive rather than negative.

A similar relationship existed amongst the Hypergastrinaemic DU patients. The correlation between plasma gastrin and somatostatin was positive, but did not reach statistical significance (Spearman's rank correlation coefficient $r_s = 0.152$, t value = 1.808 with 138 d.o.f., $p = \text{NS}$). A negative correlation between plasma gastrin and somatostatin was evident in the Normogastrinaemic patients but this did not quite reach significant levels (Spearman's rank correlation coefficient $r_s = -0.129$, t value = -1.511 with 134 d.o.f., $p = 0.068$).

7.6.5 Duodenal bulb acidity

The acid exposure within the duodenal bulb was analysed in three ways:

- a) the cumulative pH percentage at the acidic pH levels;
- b) analysis of the acid peaks to below pH 4.0;
- c) the pattern of the onset, duration and cessation of duodenal acidification. The results of the three groups of healthy subjects,

Hypergastrinaemic DU patients and Normogastrinaemic DU patients were compared.

The frequency distribution of the data points from the duodenal electrode was calculated with the cumulative pH percentage analysis, and results to pH levels 4.0, 3.0 and 2.0 were considered. Table 7.2 shows the results of this analysis. The Hypergastrinaemic patients experienced a greater exposure to acid compared to the Normogastrinaemic patients, but the differences did not quite reach statistical significance probably due to the relatively small numbers. Compared to the healthy subjects, the Hypergastrinaemic patients demonstrated an increased acid exposure at the higher acid levels of below pH 3 and 2. This increased exposure also failed to reach statistical significance.

Table 7.2: Frequency distribution of duodenal pH data after cephalic stimulation and a meal in healthy subjects (VOL), Hypergastrinaemic (H^R_G) and Normogastrinaemic (N^O_G) duodenal ulcer patients.

CUMULATIVE PERCENTAGE OF DUODENAL pH DATA*			
pH Level	VOL	H^R_G	N^O_G
<4	28.8 (0.9-78.8)	28.3 (7.4-63.5)	9.5 (2.5-39.2)
<3	17.2 (0.0-53.1)	20.4 (1.5-50.5)	4.7 (1.1-23.1)
<2	5.3 (0.0-36.7)	11.3 (0.7-29.0)	1.4 (0.1-13.5)

*Median (range)

At all levels, p value = NS (Mann-Whitney U-Test)

The analysis of the acid peaks within the duodenal bulb to below the pH level 4.0 is shown in Table 7.3, and this revealed a similar pattern of duodenal bulb acid exposure in the control and H^RG patient groups. However, the N^OG DU patients experienced not only reduced duration of acid exposure (as shown by the time spent at pH <4 and the duration of each acid peak), but also reduced numbers of acid peaks. The overall range of the numbers of acid peaks was, however, similar to the other two groups.

Table 7.3: Analysis of acid peaks in the duodenal bulb after cephalic stimulation and a meal in healthy subjects (VOL), Hypergastrinaemic (H^RG) and Normogastrinaemic (N^OG) duodenal ulcer patients.

	<u>VOL</u>	<u>H^RG</u>	<u>N^OG</u>
Time (minutes)	15.0	15.2	4.7
at pH <4	(0.1-46.7)	(4.1- 37.3)	(0.9-9.1)
Duration (min.)	2.9	3.6	2.5
per peak	(0.7-18.9)	(1.6-13.2)	(1.1-6.1)
No. of acid	3.7	3.8	1.9
peaks	(0.2-7.8)	(0.4-7.5)	(0.7-9.1)
No. of peaks	3.0	3.4	1.8
<5 minutes	(0.2-6.4)	(0.0-6.8)	(0.3-8.1)
No. of peaks	0.5	0.7	0.3
>5 minutes	(0.0-1.5)	(0.2-1.3)	(0.0-1.6)

Data standardized to one hour

At all levels, p value = NS (Mann-Whitney U Test)

The third method of analysis considered the onset, duration and cessation of duodenal acidification. The data is presented in tabular form in Table A.11.

a) Onset of duodenal acidity (Figure 7.9)

Following cephalic stimulation by modified sham feeding, the median times to the onset of duodenal acidification was similar for each of the three study groups, although the ranges of times varied widely. After ingestion of the meal, the H^{RG} and control groups showed similar times for onset of acidification, whereas the N^{OG} patients demonstrated a marked delay in the onset of duodenal acidity, this being significantly slower than the controls ($p < 0.05$, Mann-Whitney U test). The delay after the meal was significantly prolonged when compared to after the cephalic stimulation only in the H^{RG} group; presumably N^{OG} group had insufficient numbers for the range.

b) Time spent below pH 4 (Figure 7.10)

The total duration of duodenal acidity after feeding was considered in terms of the time spent below pH 4.0 following modified sham feeding and the ingested meal. The Hypergastrinaemic patients showed a more prolonged acid exposure after both feeds, whereas the N^{OG} patients experienced very little exposure to acid. The differences between the three groups did not reach significant levels.

c) Time to peak duodenal acid response (Figure 7.11)

Following the onset of cephalic stimulation, the H^{RG} patient group and controls attained peak duodenal acid levels at similar times. The N^{OG} patient group reach peak acid levels faster, but the difference did not reach significance. After the meal, however, both patient groups

EFFECT OF MSF AND A MEAL IN DU PATIENTS AND CONTROLS

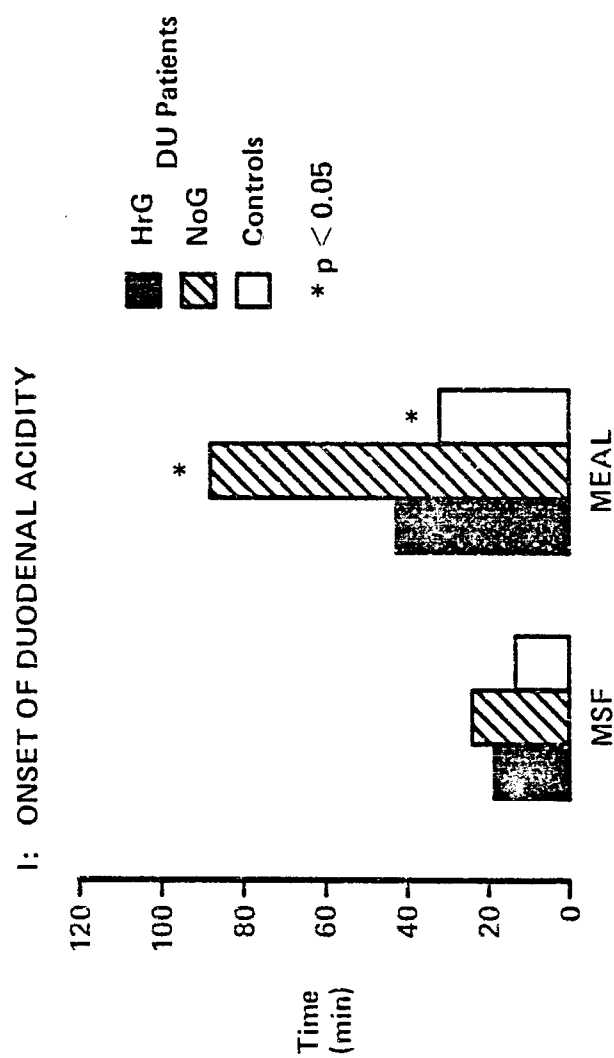


Figure 7.9

EFFECT OF MSF AND A MEAL IN DU PATIENTS AND CONTROLS

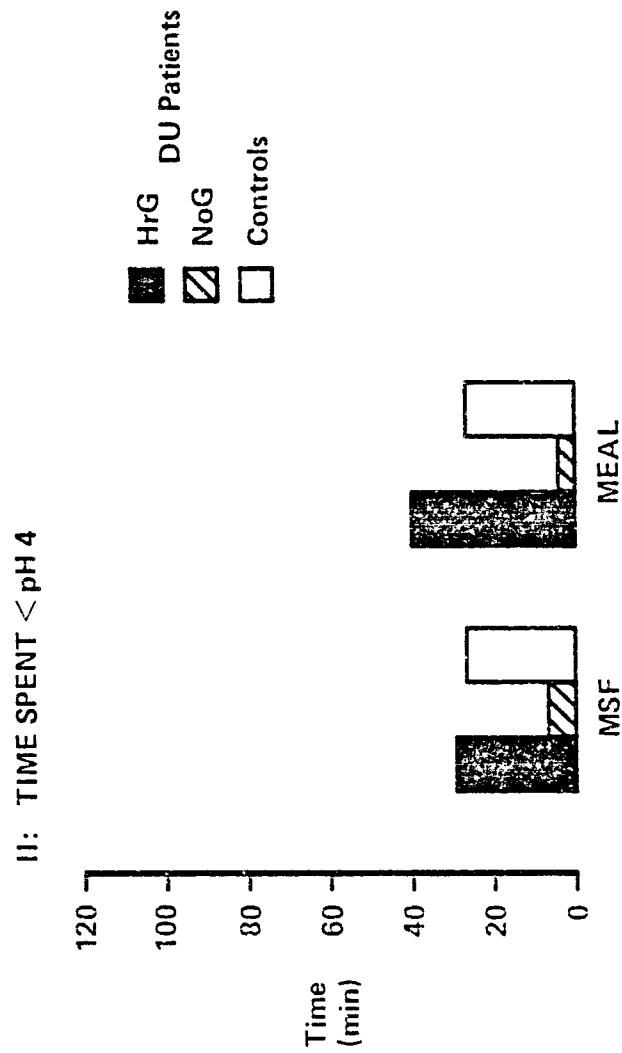


Figure 7.10

EFFECT OF MSF AND A MEAL IN DU PATIENTS AND CONTROLS

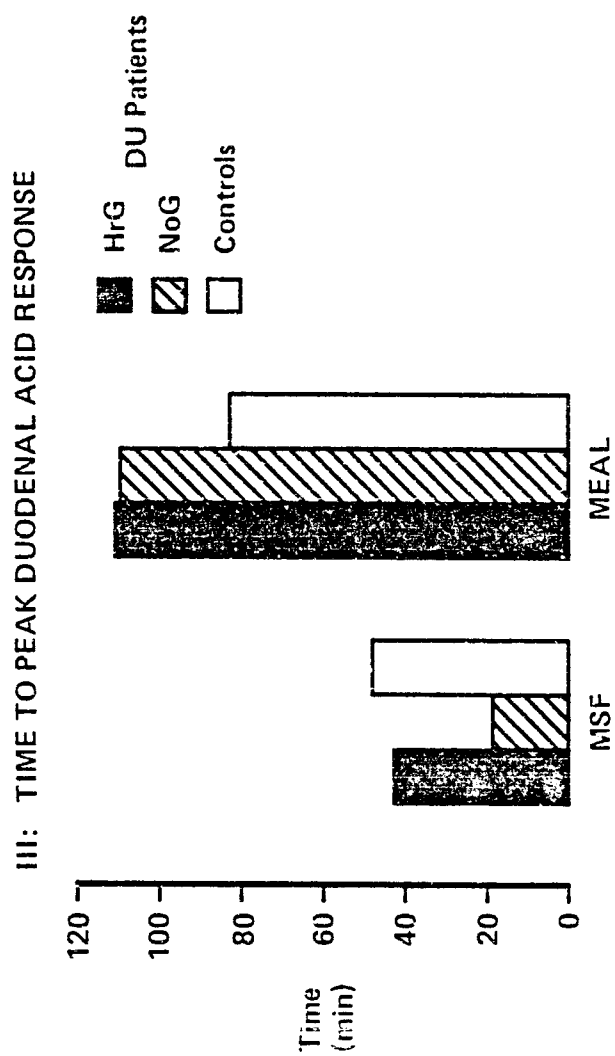


Figure 7.11

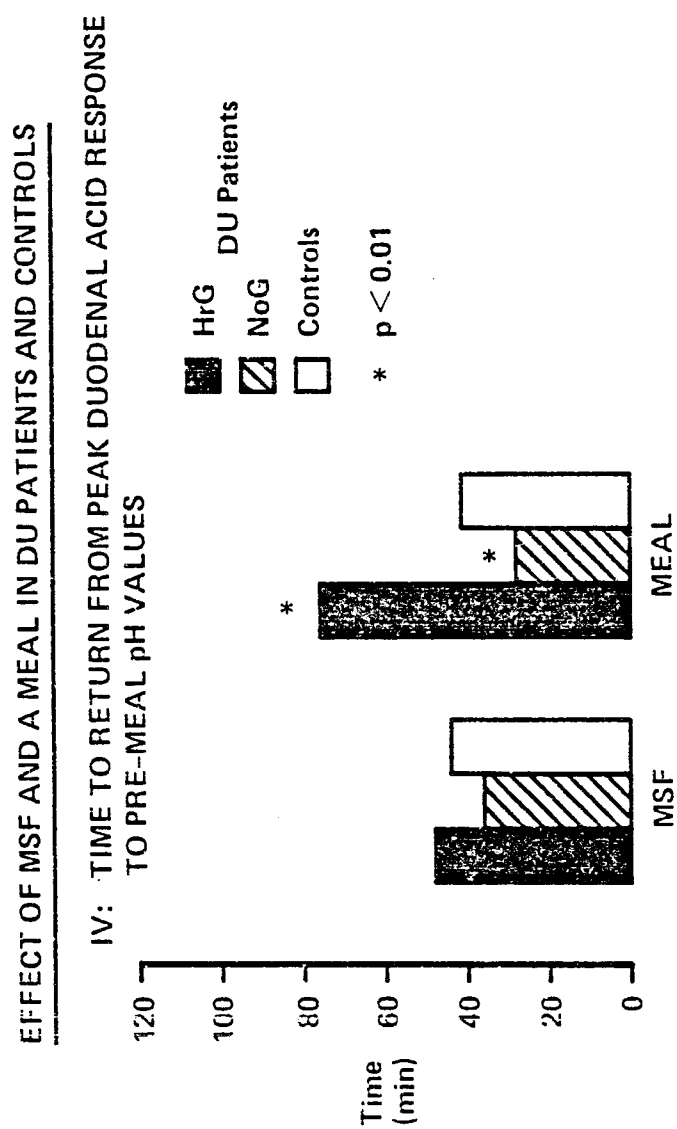


Figure 7.12

showed prolonged times compared to the control group, and again the differences were not significant, presumably because of the numbers. For all three groups, the times to peak response after the meal were significantly slower than that after cephalic stimulation (H^{RG} and N^{OG} : $p < 0.01$; VOL : $p < 0.02$, Wilcoxon Matched-Pairs Signed-Ranks test).

d) Time to return from peak duodenal acid response to pre-meal pH values (Figure 7.12)

The time taken from the peak acid response to return to the pre-meal pH value is an indirect measure of the "switch-off" mechanism of gastric acid secretion. Following cephalic stimulation, the three groups showed similar times. However, after the meal, the time to return to pre-meal pH levels was significantly slower amongst the Hypergastrinaemic patients compared to the Normogastrinaemic patients ($p < 0.01$, Mann-Whitney U test), but not quite as marked when compared to the control group. In addition, only the H^{RG} group showed a significantly slower time after the meal compared to after the sham feed.

7.7 DISCUSSION

The values for fasting plasma gastrin obtained in this study are at variance with other reports (Hansky and Cain 1969, Stadil and Rehfeld 1971, McGuigan and Trudeau 1973, Levine et al 1976). These variations are not thought to be due to population differences (Walsh 1979), although it should be noted that almost all of the subjects studied in this investigation were of Celtic origin, and Scots have been found to have an increased parietal cell mass (Lam 1984). The more likely reasons for the differences in gastrin levels are: differing

techniques of gastrin radioimmunoassay with a tendency for the newer improved assays to yield lower values (Berson and Yalow 1972); and the skewed distribution (Byrnes et al 1970, Berson and Yalow 1972) with wide ranges in the gastrin levels for patients and control subjects (Hansky and Cain 1969, Stadil and Rehfeld 1971).

In this present investigation, the patients with duodenal ulcer, as a group, exhibited significantly higher fasting plasma gastrin levels compared to the healthy subjects. This finding confirms the results of some reports (Byrnes et al 1970, Reeder et al 1970, Ganguli and Hunter 1972, Mayer et al 1974, Gedde-Dahl 1975), but is not in keeping with others (McGuigan and Trudeau 1973, Steen et al 1980, Poulsen, Eisum and Amdrup 1986). Indeed, some authors have reported fasting plasma gastrin levels of duodenal ulcer patients to be lower than controls (Korman, Soveny and Hansky 1971, Trudeau and McGuigan 1971, Feldman, Richardson and Fordtran 1980). These differing reports illustrate that there is considerable overlap in the gastrin levels not only between duodenal ulcer patients and healthy subjects, but also between the DU patients themselves. The distribution of fasting plasma gastrin data was not normal, and transformation by natural logarithms normalised the distribution. This has been observed previously (Ardill 1973).

It is well recognized that cephalic stimulation via the vagus induces a strong gastric acid secretory response (Richardson et al 1977, Konturek et al 1978, Jarhult, Graffner and Bloom 1984). However, the response of plasma gastrin to such stimulation has not been consistently reported. Some studies has not shown any appreciable increase in plasma gastrin following modified sham feeding (Feldman et al 1979, Stenquist, Rehfeld and Olbe 1979, Konturek et al 1981,

Jarhult, Graffner and Bloom 1984, Kohn et al 1985), while others have demonstrated a significant rise over basal levels (Mayer et al 1974, Knutson, Olbe and Ganguli 1974, Feldman, Richardson and Fordtran 1980). In the present study, both the control subjects and the patients with duodenal ulcer failed to show a significant increase in plasma gastrin over basal levels, after cephalic stimulation. These results would suggest that vagally-released gastrin is not a significant contributor to the stimulation of gastric acid secretion in either health or duodenal ulcer disease. The roles of somatostatin and duodenal acidification (via secretin) in inhibiting gastrin release are discussed below.

Following the ingested meal, the plasma gastrin levels were significantly raised in both the controls (by 180.0%) and DU patients (by 318.8%); these results being well established (Byrnes et al 1970, Hayes et al 1973, Mayer et al 1974, Fritsch, Hausaman and Rick 1976, Richardson et al 1977). The response amongst the patients was significantly stronger than the controls and, in addition, the gastrin levels of the DU patients remained significantly higher than those of the controls. It would, therefore, appear that patients with duodenal ulcer do have excessive basal and post-stimulation levels of plasma gastrin.

However, closer examination of the fasting gastrin data in this study showed there to be a bimodal distribution (Figure 7.2), and two clearly defined populations of duodenal ulcer patients appeared to exist. The "Hypergastrinaemic" patients exhibited exaggerated gastrin responses to both cephalic stimulation and the ingestion of the meal. On the other hand, the "Normogastrinaemic" patients demonstrated gastrin responses that approximated to those of the healthy subjects.

Similar hypergastrinaemic patients have previously been noted (Berson and Yalow 1972, Mayer et al 1974, Montoro, Garcia and Samitier 1985), but not fully described. In contrast to patients with Zollinger-Ellison syndrome who experience no rise in plasma gastrin after feeding (Berson and Yalow 1972, Malagelada 1977), these hypergastrinaemic DU patients exhibited marked and significant increases in plasma gastrin after both ingestion of a meal and cephalic stimulation. The exaggerated response to cephalic stimulation suggests a vagal hyperactivity in these patients.

The triad of duodenal ulceration, hypergastrinaemia and hyperacidity has been described (Hansky 1977, Walsh 1977). The usual causes in unoperated patients are gastrinoma, hyperparathyroidism, antral G cell hyperplasia and, in some cases, gastric outlet obstruction (Ebeid and Fisher 1976). The increased gastrin response to feeding is thought to be due to increased gastrin stores, increased number of G cells (Fiddian-Green and Vinik 1983), some autonomy in release of gastrin (Hansky 1979), or a diminished inhibition of gastrin release by antral acid (Wormsley 1979). Indeed, high gastrin levels in the presence of hyperacidity in ulcer patients favours the latter explanation. This defective inhibition of gastrin release was confirmed in a study from Denmark (Steen et al 1980) in which a significant negative correlation was demonstrated between gastrin levels and acid output in controls but not in duodenal ulcer patients. It has also been suggested that these patients with hypergastrinaemia, hyperacidity and no gastrin-producing tumour, have an over-sensitive or overactive G cell-bearing antro-duodenal mucosa (Berson and Yalow 1972).

The "Normogastrinaemic" ulcer patients had fasting gastrin levels similar to the healthy subjects, and likewise experienced no increased

response to cephalic stimulation. This group of ulcer patients may conceivably represent the majority of "ordinary" DU patients, thereby confirming findings of earlier reports (Konturek et al 1978, Stenquist, Rehfeld and Olbe 1979, Kisfalvi, Foldvari and Szucs 1983). The meal-stimulated peak gastrin response in these patients was also similar to that exhibited by the controls. Similar results have been found by other workers (McGuigan and Trudeau 1973, Stern and Walsh 1973).

The reported (McGuigan and Trudeau 1973, Walsh and Grossman 1975b, Wormsley 1979) sustained increased levels of post-prandial plasma gastrin were only observed, in this study, amongst the subset of H^RG patients. This would confirm that it is this subset of duodenal ulcer patients that have a defective inhibition of gastrin release, whereas the N^OG patients react in similar fashion to healthy subjects. In a study on the mechanism of inhibition of gastric acid secretion, Sainz and co-workers (1987) distinguished two groups of DU patients, one group who showed a clearly defective inhibition. It would be interesting to investigate this group further by measuring their plasma gastrin responses.

The fasting plasma secretin levels were not significantly different between healthy controls and the two groups of DU patients. As with the plasma gastrin levels, the secretin levels of DU patients have previously been reported as being significantly higher than (Chey, Hendricks and Tai 1977), significantly lower than (Ward and Bloom 1974, McLoughlin, Green and Buchanan 1978), and similar to controls (Greenberg et al 1982, Kurokawa et al 1982). The distribution of fasting data, however, was near-normal, log-transformation did not add any benefit. The data has been logged by other authors prior to analysis (Greenberg et al 1982). It has been shown that, following

duodenal acidification, plasma secretin levels rise briefly (Greenberg et al 1982, Jorde and Burhol 1985), only to fall during the first post-prandial hour. In the present study, only the Hypergastrinaemic patients showed any appreciable increase in plasma secretin after duodenal acidification by either cephalic stimulation or a meal. As the post-prandial plasma gastrin levels were prolonged in these patients and neither the controls nor N^OG patients exhibited rises in plasma secretin after the meal, the physiological role of secretin in inhibiting gastrin release in Man is questionable. This proposal is supported by the significant negative correlation demonstrated between plasma gastrin and secretin in the healthy subjects and the N^OG DU patients. The doubts concerning the role of secretin in Man have previously been raised (Ward and Bloom 1974, Kleibeuker et al 1984), although its case has been proved in dogs (Johnson and Grossman 1968). That duodenal acidification inhibits gastrin-stimulated gastric acid secretion (Wormsley 1971, Sainz et al 1985) is not disputed, although this inhibition is defective in some duodenal ulcer patients (Shay et al 1942, Walsh, Richardson and Fordtran 1975, Malagelada et al 1977, Sainz et al 1985). The presence of a second hormone to fill this role has been sought, and an inhibitory agent called bulbogastrone has been proposed (Uvnas 1971).

The fasting plasma somatostatin levels shown in this study were similar to those reported in some studies (Colturi, Unger and Feldman 1984, Lucey et al 1985) but were lower than in others (Gustavsson et al 1982, Lucey et al 1984, Kishimoto et al 1985). The Normogastrinaemic patients exhibited the highest levels, being significantly greater than both the H^RG DU patients and controls. The influence of the vagus on the somatostatin release was confirmed in part by the responses to cephalic stimulation, as only the duodenal

ulcer patients experienced rises in plasma somatostatin after such stimulation; this being significant amongst the N^OG group. Previous studies (Colturi, Unger and Feldman 1984, Lucey et al 1985, Calabro et al 1986) have demonstrated significant post-prandial somatostatin rises in normal subjects but not in DU patients. However, in the present study, the meal response was markedly raised and prolonged in the N^OG group, while the H^RG patients and controls exhibited minimal responses. There was no significant correlation between post-stimulation plasma gastrin and somatostatin levels in either the healthy subjects or DU patient groups. However, the correlation tended to be negative in the N^OG DU patients which was unexpected.

The higher plasma somatostatin levels experienced by the Normogastrinaemic DU patients might explain their relatively lower plasma gastrin levels and responses. Conversely, the differences in somatostatin responses might be explained on the basis of the Hypergastrinaemic DU patients exhibiting a defect in somatostatin-induced inhibition of plasma gastrin release. The foregoing findings of this investigation would favour the latter explanation.

Gastric emptying has been reported to be increased in duodenal ulcer patients (Lagerlof, Rudewald and Perman 1960, Griffith et al 1968, McLoughlin, Green and Buchanan 1978), but this has not been confirmed by a recent report (Holt et al 1986) in which such patients were studied before and after medical treatment. The present study confirmed this latter finding, and provided evidence to the contrary. The onset of duodenal acidity was longer in both patient groups after cephalic stimulation and even slower following the ingested meal. Likewise, the time to peak duodenal acid response after the meal was slower in both patient groups. This would suggest a hold-up in the

normal emptying of gastric acid from the stomach in duodenal ulcer patients, and this appears to be more pronounced in the Normogastrinaemic sub-group of patients.

Following both cephalic stimulation and ingestion of a meal, the H^{RG} patients exhibited a delay in the return to pre-meal pH levels. The possible reasons for this observation are: these patients may have a defect in the neutralizing mechanisms within the duodenal bulb; the clearing mechanism of the antro-duodenal segment may be defective; and these Hypergastrinaemic patients have a defect in the "switch-off" mechanism of gastric acid secretion. The data from presented in this study, including the sustained gastrin release, favour the last explanation.

In conclusion, vagally-released gastrin does not appear to be a significant contributor to the stimulation of gastric acid secretion in either health or duodenal ulcer disease. The results of this study show that duodenal ulcer patients, as a group, have excessive basal and post-stimulation levels of plasma gastrin. There is, however, a clearly defined sub-group of duodenal ulcer patients, the "Hypergastrinaemic" patients who respond in a different manner compared to the "ordinary" duodenal ulcer patients. These H^{RG} DU patients demonstrate: significantly greater increases in plasma gastrin in response to cephalic stimulation and a meal; vagal hyperactivity; defective inhibition of gastrin release by both antral acidification and via somatostatin; and an impaired "switch-off" mechanism of gastric acid secretion. In addition, the physiological role of secretin in inhibiting gastrin release in Man is questionable.

CHAPTER 8

CONCLUSIONS AND THE FUTURE

8.1 DUAL GASTRO-DUODENAL pH MONITORING SYSTEM

The pH monitoring system developed and described in this investigation produced a reliable and accurate recording of the antral and duodenal bulb pH. The numerous problems encountered in the past, which hampered previous studies, have largely been overcome with the advent of improved pH monitoring equipment and recording techniques. The small glass pH electrodes are the most suitable for intra-luminal pH monitoring and are accurate over long periods of time as they do not exhibit a significant drift in response over 24 hours. If such drift does occur and requires correction, it is generally linear and readily correctable by the appropriate computer software. The major problem of placement of the duodenal electrode at a known and reproducible distance from the pylorus, can be overcome by the use of a weighted bag attached to the electrode. The peristaltic pull on the bag, in the region of the duodeno-jejunal flexure, against fixation of the electrode wires at the subject's mouth, maintains the position of the electrode within the bulb. However, accurate placement is only possible towards the apex of the bulb. Siting the electrode in this manner at the base of the bulb is liable to be complicated by prolapse of the electrode into the stomach. Rune's method (Rune 1981) of utilizing a "chain" of four electrodes overcomes this problem but it does not appear to be suitable for long-term monitoring.

The computer-based storage and analysis allowed for large amounts of data to be recorded, and detailed assessment of the respective pH patterns was possible. New modified software programmes provided additional analysis of the peaks of acidity within the duodenal bulb which described the pattern of motility of the antro-duodenal segment.

8.2 GASTRO-DUODENAL pH PROFILE IN HEALTH

The profile of gastric pH in the cohort of healthy subjects was steady throughout the duration of the 24 hour study, except for rises in pH levels that occurred at the times of eating and drinking. A small percentage of normals experienced spontaneous rises in pH in the early hours of the morning, and these rises were possibly related to duodenogastric reflux.

The pH within the duodenal bulb demonstrated the frequent fluctuations that have been noted in previous studies. The profile of daytime acid exposure exhibited an exponential curve and that of nocturnal exposure was similar. Of note, was the similar exposure to acid during both day and night, although there was a slight tendency for the daytime exposure to be greater. The pattern of acid peaks within the bulb was also similar during day and at night. The effect of gastric stimulation produced only a slight increase in the acid exposure in the bulb.

8.3 GASTRO-DUODENAL pH PROFILE IN DUODENAL ULCER DISEASE

Patients with active duodenal ulcer exhibited a profile of gastric pH over the 24 hours that was similar to the healthy subjects. Spontaneous rises in pH were also noted at night in a small percentage of patients.

The fasting duodenal bulb pH of the DU patients was similar to that of the normals, and the fluctuating pattern was also noted. The daytime exposure to acid showed the same exponential profile as the controls and no significant difference was observed. A similar picture was shown for the nocturnal acid exposure, and, contrary to earlier reports,

the nocturnal exposure was not significantly greater than that experienced during the day. Gastric stimulation produced the same effect as seen in the healthy subjects, but the analysis of the acid peaks suggested a defect in the propulsive duodenal motility in the DU patients. These results suggest that gastric acid is not of primary pathophysiological importance in duodenal ulcer disease.

8.4 THE CONTROLS OF GASTRIC ACID SECRETION

The responses of plasma gastrin, secretin and somatostatin and the concurrent duodenal bulb pH changes to the cephalic and gastric phases of gastric acid secretion have demonstrated some important pathophysiological abnormalities present in duodenal ulcer patients. These patients exhibited excessive basal and post-stimulation plasma gastrin levels. A clearly defined subset of duodenal ulcer patients existed, the "Hypergastrinaemic" patients, who behaved in a different fashion compared to the "ordinary" "Normogastrinaemic" patients and they demonstrated a number of abnormalities: an exaggerated response to cephalic stimulation, suggesting vagal hyperactivity; a defect in the inhibitory mechanism of gastrin release, which appeared to be due to an impaired somatostatin response; and a defect in the negative feedback of duodenal acidification on gastric acid secretion. In addition, the vagally-released gastrin did not appear to be a significant contributor to the stimulation of gastric acid secretion in either health or duodenal ulcer disease. The results also suggest that the physiological role of secretin in inhibiting gastrin release in Man is questionable.

8.5 THE FUTURE

The use of the dual gastro-duodenal pH monitoring system is, at this stage of development and investigation, more for research work, rather than to be used as a diagnostic tool. However, as reduction of gastric acid levels produces healing of duodenal ulcers, the next study suited for the system would be assessment of duodenal acidity following acid reduction therapy. As a corollary, the efficacy of newer drugs can be assessed. This would yield more meaningful results in documenting the change in duodenal acidity following medication. At present, these studies are being performed by monitoring 24 hour intra-gastric pH only (Walt et al 1981, Walt et al 1983, Etienne et al 1985).

The plasma gastrin results have shown that there is a definite subset of duodenal ulcer patients that respond quite differently to gastric stimulation. It might prove beneficial to identify these patients as they may prove to be in the group of non-responders. It seems feasible therefore, after many patients have been studied, to construct a list of predictive parameters of those patients who might not respond to therapy, or those who might benefit from early surgery.

A further area of study with this monitoring system is the condition of duodeno-gastric reflux. In this regard, the system may be adapted for clinical testing. In addition, the receiving unit has space for two further input sources. These may be added and adapted to monitor intra-luminal pressure changes. Thus, the collected data of simultaneously measured gastro-duodenal pH and intra-luminal pressure changes would be of importance in furthering the understanding of the physiology of the gastro-duodenal segment.

APPENDIX

Table A.1: Cumulative percentage of total 24 hour duodenal pH recordings in duodenal ulcer patients and healthy subjects.

CUMULATIVE % OF TOTAL DUODENAL pH RECORDINGS*

pH	time	Controls	DU Patients
<5	day	39.6 (7.1-80.9)	37.8 (3.7-68.8)
	night	37.7 (12.6-71.4)	34.5 (0.0-85.6)
<4	day	23.9 (1.0-60.6)	24.8 (1.2-51.3)
	night	25.4 (6.9-65.9)	22.2 (0.0-74.6)
<3	day	14.6 (0.1-42.2)	11.7 (0.4-39.2)
	night	12.8 (2.2-61.0)	12.5 (0.0-57.2)
<2	day	8.4 (0.0-31.8)	3.3 (0.0-29.9)
	night	4.5 (0.2-42.6)	5.0 (0.0-35.8)
<1	day	0.3 (0.0-15.0)	0.1 (0.0-9.1)
	night	0.5 (0.0-28.5)	0.1 (0.0-22.2)

*median (range)

At all levels, p = NS, Mann=Whitney U Test

Table A.2: Analysis of number of acid peaks of varying duration within the duodenal bulb in controls and duodenal ulcer patients.

a) CONTROLS: NUMBER OF PEAKS**

Duration (min.)	shorter than		longer than		p* short/long	
	Day	Night	Day	Night	day	night
1	1.2 (0.3-1.7)	0.7 (0.2-1.8)	2.8 (1.3-6.1)	2.5 (2.1-4.4)	<0.01	<0.01
2	2.4 (0.7-4.6)	2.2 (0.7-3.4)	1.1 (0.3-4.1)	1.5 (0.6-3.0)	<0.02	NS
3	2.6 (0.8-3.7)	2.6 (0.8-5.2)	0.9 (0.1-3.1)	0.9 (0.5-2.0)	<0.01	<0.05
4	2.8 (0.9-5.4)	3.0 (0.8-4.1)	0.6 (0.1-2.5)	0.8 (0.3-1.8)	<0.01	<0.02
5	2.9 (0.1-6.2)	3.2 (0.4-4.1)	0.5 (0.0-2.2)	0.6 (0.0-1.5)	<0.01	<0.01

b) DUODENAL ULCER PATIENTS:

1	0.9 (0.3-2.2)	0.5 (0.3-2.7)	3.1 (0.9-5.6)	1.7 (0.2-5.1)	<0.01	NS
2	2.1 (1.1-4.3)	1.1 (0.4-6.1)	1.8 (0.2-3.4)	1.0 (0.1-2.2)	NS	NS
3	2.5 (1.1-5.7)	1.2 (0.4-6.7)	1.4 (0.2-2.0)	0.7 (0.0-1.8)	<0.01	NS
4	2.9 (1.1-6.0)	1.4 (0.4-6.7)	1.1 (0.1-1.6)	0.7 (0.0-1.4)	<0.01	NS
5	2.9 (0.0-8.2)	2.9 (0.0-7.1)	0.7 (0.0-2.0)	0.7 (0.0-2.4)	<0.01	<0.01

**Medians, ranges; data standardised to one hour

*Wilcoxon Matched-Pairs Signed Ranks test

Table A.3: Cumulative pH percentage of gastric pH data in patients with duodenal ulcer.

CUMULATIVE PERCENTAGE* OF TOTAL GASTRIC pH DATA			
pH LEVEL	DAY	NIGHT	p VALUE **
>1	99.9 (59.7 - 100.0)	100.0 (27.6 - 100.0)	NS
>2	52.5 (18.1 - 97.9)	52.4 (0.3 - 94.4)	NS
>3	20.9 (7.0 - 45.1)	1.5 (0.0 - 49.0)	<0.001
>4	12.7 (3.2 - 29.8)	0.75 (0.0 - 38.5)	<0.001
>5	4.8 (0.8 - 14.7)	0.1 (0.0 - 29.9)	<0.001
>6	2.0 (0.1 - 7.5)	0.0	NS

Table A.4: Duration of rises in intragastric pH over 24 hours in healthy subjects and patients with duodenal ulcer.

		CONTROLS		DU PATIENTS	
		Time (min.)*	p value"	Time (min.)*	p value"
pH >1	day	59.2 (36.4-59.7)	NS	59.3 (20.2-59.7)	NS
	night	59.9 (28.2-59.9)		59.6 (10.9-60.0)	
>2	day	39.4 (10.2-58.2)	NS	26.6 (9.8-52.1)	<0.05
	night	24.6 (2.4-59.9)		22.3 (0.0-54.7)	
>3	day	15.1 (6.3-30.7)	<0.01	11.9 (3.9-25.5)	<0.01
	night	6.8 (0.1-14.1)		0.8 (0.0-28.8)	
>4	day	6.7 (1.9-21.8)	<0.01	7.1 (1.8-16.8)	<0.01
	night	1.6 (0.0-9.7)		0.3 (0.0-21.9)	
>5	day	1.7 (0.1-17.0)	NS	2.4 (0.3-8.0)	<0.01
	night	1.0 (0.0-5.7)		0.0 (0.0-16.8)	

*Median, ranges; data standardized to one hour

"Wilcoxon Matched-Pairs Signed-Ranks test

TABLE A.5: Plasma gastrin responses to modified sham feeding (MSF) and a meal in duodenal ulcer patients and healthy subjects.

PLASMA GASTRIN (ng/l) (median, range)			
Time*	DU Patients(n=22)	Controls(n=20)	p**value
-1.00	65.0 (20-100)	22.5 (10-80)	
-0.30	55.0 (20-105)	20.0 (5-90)	
0.00	50.0 (20-100)	25.0 (5-110)	
0.10	60.0 (20-185)	25.0 (5-120)	
0.20	50.0 (20-170)	20.0 (5-200)	
0.30	70.0 (20-200)	22.5 (5-140)	<0.001
0.45	55.0 (20-160)	22.5 (5-130)	
1.15	52.5 (20-110)	22.5 (5-90)	
2.00	40.0 (20-80)	25.0 (5-70)	
2.30	40.0 (20-90)	25.0 (50-70)	
2.40	60.0 (15-180)	37.5 (10-240)	
2.50	120.0 (20-285)	70.0 (20-300)	
3.00	167.5 (20-325)	60.0 (20-160)	<0.001
3.15	150.0 (20-310)	55.0 (20-350)	
3.45	100.0 (30-270)	40.0 (20-240)	
4.30	100.0 (20-175)	45.0 (20-200)	
5.30	65.0 (20-130)	40.0 (10-210)	

*Time of sampling (hr,min)

**Mann-Whitney U Test: At all levels, a significant difference (min. <0.01) observed between the two groups

TABLE A.6: Plasma gastrin responses to modified sham feeding (MSF) and a meal in "Hypergastrinaemic" (H^RG) and "Normogastrinaemic" (N^OG) duodenal ulcer patients.

PLASMA GASTRIN (ng/l) (median, range)			
Time"	H ^R G* (n=11)		N ^O G** (n=11)
-1.00	80.0 (50-100)		27.5 (20-65)
-0.30	60.0 (50-105)	Fasting	30.0 (20-65)
0.00	70.0 (50-100)		30.0 (20-60)
0.10	80.0 (60-185)		30.0 (20-50)
0.20	70.0 (65-170)	MSF	30.0 (20-50)
0.30	90.0 (70-200)		35.0 (20-60)
0.45	77.5 (55-160)		35.0 (20-50)
1.15	65.0 (45-110)		25.0 (20-55)
2.00	62.5 (40-80)		25.0 (20-40)
2.30	55.0 (30-90)		30.0 (20-60)
2.40	75.0 (60-180)		42.5 (15-70)
2.50	200.0 (100-285)	MEAL	87.5 (20-120)
3.00	225.0 (150-325)		87.5 (20-175)
3.15	175.0 (110-310)		92.5 (20-175)
3.45	125.0 (70-270)		75.0 (30-120)
4.30	125.0 (75-175)		60.0 (20-115)
5.30	100.0 (50-130)		52.5 (20-80)

*Hypergastrinaemic DU patients

**Normogastrinaemic DU patients

"Time of sampling (hr,min)

TABLE A.7: Plasma secretin responses to modified sham feeding (MSF) and a meal in duodenal ulcer patients and healthy subjects.

PLASMA SECRETIN (ng/l) (median, range)				
Time*	DU Patients(n=22)		Controls(n=20)	p** value
-1.00	20.0 (5-75)		25.0 (5-50)	
-0.30	25.0 (0-50)	Fasting	25.0 (10-60)	
0.00	25.0 (0-60)		25.0 (10-55)	
0.10	20.0 (5-100)		25.0 (10-40)	
0.20	25.0 (5-55)	MSF	25.0 (5-45)	
0.30	25.0 (0-55)		20.0 (5-45)	
0.45	30.0 (5-60)		25.0 (5-50)	NS
1.15	27.5 (10-62)		25.0 (10-60)	
2.00	25.0 (5-60)		25.0 (10-55)	
2.30	25.0 (5-45)		30.0 (10-45)	
2.40	25.0 (5-40)		30.0 (10-45)	
2.50	25.0 (10-55)	MEAL	25.0 (5-50)	
3.00	22.5 (5-65)		20.0 (10-45)	
3.15	25.0 (5-55)		20.0 (5-40)	NS
3.45	20.0 (5-60)		17.5 (5-35)	
4.30	25.0 (5-85)		20.0 (5-65)	
5.30	25.0 (5-40)		22.5 (5-55)	

*Time of sampling (hr.min)

**Mann-Whitney U Test: at all levels, no significant differences noted

TABLE A.8: Plasma secretin responses to modified sham feeding (MSF) and a meal in "Hypergastrinaemic" (H^RG) and "Normogastrinaemic" (N^OG) DU patients.

PLASMA SECRETIN (ng/l) (median, range)			
Time*	H ^R G (n=11)		N ^O G (n=11)
-1.00	20.0 (15-75)		25.0 (5-40)
-0.30	25.0 (10-50)	Fasting	20.0 (0-50)
0.00	25.0 (10-60)		25.0 (0-40)
0.10	25.0 (5-100)		20.0 (5-35)
0.20	30.0 (10-55)	MSF	25.0 (5-40)
0.30	25.0 (15-55)		25.0 (0-45)
0.45	32.5 (15-60)		30.0 (5-50)
1.15	30.0 (10-62)		20.0 (10-50)
2.00	25.0 (15-60)		25.0 (5-45)
2.30	25.0 (15-45)		27.5 (5-40)
2.40	25.0 (5-40)		25.0 (5-40)
2.50	30.0 (10-55)	Meal	22.5 (10-50)
3.00	25.0 (10-65)		20.0 (5-50)
3.15	30.0 (15-55)		25.0 (5-40)
3.45	20.0 (10-60)		20.0 (5-45)
4.30	25.0 (10-85)		25.0 (5-40)
5.30	25.0 (10-35)		25.0 (5-40)

*Time of sampling (hr,min)

At all levels, no statistical difference observed (Mann-Whitney U-Test)

TABLE A.9: Plasma somatostatin responses to modified sham feeding (MSF) and a meal in duodenal ulcer patients and healthy subjects.

PLASMA SOMATOSTATIN (ng/l) (median, range)				
Time*	DU Patients(n=22)		Controls(n=20)	p**value
-1.00	15.0 (2-55)		10.0 (2-60)	
-0.30	16.5 (3-60)	Fasting	10.5 (2-105)	
0.00	15.0 (1-90)		9.0 (1-60)	
0.10	20.0 (2-125)		10.0 (2-60)	<0.05
0.20	15.0 (1-75)	MSF	9.0 (1-65)	<0.05
0.30	14.0 (6-75)		10.0 (2-65)	<0.05
0.45	15.0 (1-85)		10.0 (1-60)	
1.15	10.0 (2-75)		11.0 (2-68)	
2.00	12.5 (2-70)		10.0 (2-45)	
2.30	11.0 (1-140)		10.5 (2-45)	
2.40	10.0 (2-130)		10.0 (2-70)	
2.50	15.5 (2-140)	Meal	7.5 (2-65)	
3.00	11.0 (1-90)		10.0 (2-45)	
3.15	13.5 (2-140)		10.0 (2-50)	
3.45	14.0 (2-130)		10.0 (1-50)	
4.30	12.5 (1-135)		9.0 (2-35)	
5.30	11.0 (2-110)		9.0 (1-40)	

*Time of sampling (hr,min)

**Mann-Whitney U Test

TABLE A.10: Plasma somatostatin responses to modified sham feeding (MSF) and a meal in "Hypergastrinaemic" (H^RG) and "Normogastrinaemic" (N^OG) duodenal ulcer patients.

PLASMA SOMATOSTATIN (ng/l) (median, range)				
Time*	H ^R G (n=11)		N ^O G (n=11)	p**value
-1.00	15.0 (2-50)		13.0 (7-55)	
-0.30	15.0 (3-50)	Fasting	19.0 (10-60)	
00.00	15.0 (1-90)		17.0 (9-60)	
00.10	20.0 (2-125)		28.0 (5-65)	
00.20	10.0 (1-75)	MSF	24.0 (10-60)	<0.05
00.30	14.5 (6-75)		12.0 (7-75)	
00.45	12.5 (1-80)		20.0 (10-85)	
01.15	10.0 (2-45)		16.0 (7-75)	
02.00	9.0 (2-70)		22.0 (10-70)	
02.30	8.5 (1-140)		17.0 (8-60)	
02.40	6.0 (2-130)		27.5 (6-85)	
13.50	7.0 (2-140)	Meal	40.0 (6-60)	
14.00	9.5 (1-25)		25.0 (9-90)	
14.15	9.5 (2-140)		22.5 (7-60)	
14.45	12.0 (2-130)		29.5 (5-75)	
15.30	9.0 (1-135)		20.0 (4-65)	
16.30	10.0 (2-110)		12.0 (2-70)	

*Time of sampling

**Mann-Whitney U Test

Table A.11: Effect of cephalic stimulation (MSF) and a meal on duodenal pH in Hypergastrinaemic (H^RG) and Normogastrinaemic (N^OG) DU patients and controls.

I: Onset of duodenal acidity

II: Time spent below pH 4

	ONSET* OF DUODENAL ACIDITY		TIME* AT pH <4	
	MSF	MEAL	MSF	Meal
H ^R G	18.5	42.0	21.0	40.2
	(3.0 - 34.0)	(3.0 - 131.0)	(0.0 - 85.0)	(0.0 - 145.6)
N ^O G	24.0	89.0	7.2	4.1
	(1.0 - 64.0)	(24.0 - 147.0)	(0.0 - 68.3)	(0.0 - 53.0)
VOL	14.0	32.0	27.6	27.1
	(1.0 - 78.0)	(3.0 - 101.0)	(0.0 - 88.0)	(0.0 - 168.6)
p** value	N ^O G/VOL			
	<0.05			

*Minutes (median, range)

**Mann-Whitney U test

Table A.12: Effect of cephalic stimulation (MSF) and a meal on duodenal pH in Hypergastrinaemic (H^{RG}) and Normogastrinaemic (N^{OG}) DU patients and controls.

III: Time to peak duodenal acid response

IV: Time from peak acid response to return to pre-meal pH levels

	TIME* TO PEAK RESPONSE		TIME* TO PRE-MEAL pH	
	MSF	Meal	MSF	Meal
H ^{RG}	42.5	111.1	48.4	76.2
	(17.1 - 77.0)	(32.0 - 152.0)	(13.7 - 100.5)	(25.0 - 114.0)
N ^{OG}	18.5	108.1	35.5	33.0
	(9.0 - 68.0)	(71.0 - 175.0)	(9.0 - 115.0)	(20.0 - 72.7)
VOL	47.6	82.3	44.9	42.0
	(9.0 - 109.0)	(17.0 - 207.0)	(7.0 - 140.0)	(10.7 - 122.0)
p**value				H ^{RG} /N ^{OG}
				<0.01

*Minutes (median,range)

**Mann-Whitney U test

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